

SPECIFICATION-MARKUP COPY

A markup copy of page 6 is attached as a separate page. Please substitute the following paragraph for the first paragraph under Background of the Invention beginning at line 16 on page 1 of the Specification:

Plant biomass as a source of energy production can include agricultural and forestry products, associated by-products and waste, municipal solid waste, and industrial waste. In addition, over 50 million acres in the United States are currently available for biomass production, and there are a number of terrestrial and aquatic crops grown solely as a source for biomass (A Wiselogle, et al. Biomass feedstocks resources and composition[.]—[I]in CE Wyman, ed. Handbook on Bioethanol: Production and Utilization. Washington, DC: Taylor & Francis, 1996, pp 105-118). Biofuels produced from biomass include ethanol, methanol, biodiesel, and additives for reformulated gasoline. Biofuels are desirable because they add little, if any, net carbon dioxide to the atmosphere and because they greatly reduce ozone formation and carbon monoxide emissions as compared to the environmental output of conventional fuels. (P Bergeron. Environmental impacts of bioethanol[.]—[I]in CE Wyman, ed. Handbook on Bioethanol: Production and Utilization. Washington, DC: Taylor & Francis, 1996, pp 90-103)[.].

AMENDED CLAIMS—MARKUP COPY

1. (Amended) A composition comprising a substantially purified thermostable [AvIII] AviIII peptide, said AviIII peptide comprising a catalytic domain of a glycosyl hydrolase family [GH]74 enzyme and a carbohydrate binding domain (CBD) III.
2. The composition of claim 1 wherein the thermostable AviIII peptide is further defined as comprising a linker and a signal sequence.
3. (Amended) The composition of claim 1 or 2 wherein the [GH] glycosyl hydrolase family 74 enzyme catalytic domain of the thermostable AviIII peptide is further defined as having a length of about 730 to about 760 amino acids.
4. (Amended) The composition of claim 1, 2, or 3[, or 4] wherein the carbohydrate binding domain (CBD) III of the thermostable AviIII peptide is further defined as comprising a length of about 80 to about 150 amino acids.
5. (Amended) The composition of claim 1,2,or 3[, or 4] wherein the carbohydrate binding domain (CBD) III of the thermostable AviIII peptide is further defined as comprising a length of about 90 amino acids.
6. (Amended) The composition of claim 3 wherein the [GH] glycosyl hydrolase family 74 enzyme catalytic domain is further defined as a polypeptide sequence of SEQ ID NO: 3.
7. (Amended) The composition of claim [4] 3 wherein the carbohydrate binding domain (CBD) III is further defined as a polypeptide sequence of SEQ ID NO: 4.

8. (Amended) The composition of claim [4] 3 wherein the carbohydrate-binding domain (CBD) III is further defined as comprising the polypeptide sequence of SEQ ID NO: 5.

9. (Amended) The composition of claim 1 wherein said AviIII protein comprises the polypeptides represented by [further defined as comprising a sequence of] SEQ ID NO: 3 and SEQ ID NO: 4.

10. (Amended) The composition of claim 1 wherein said AviIII protein is encoded by [further defined as comprising] a nucleic acid sequence having at least about 70% sequence identity to the polynucleotide sequence of SEQ ID NO: 2.

11. (Amended) The composition of claim 1 wherein said AviIII protein is encoded by [further defined as comprising] a nucleic acid sequence having at least about 80% sequence identity to the polynucleotide sequence of SEQ ID NO: 2.

12. (Amended) An isolated [A] thermostable AviIII peptide having a polypeptide sequence of SEQ ID NO: 1.

13. (Amended) The isolated thermostable AviIII peptide of claim 12 encoded by the [polypeptide further defined as having a] polynucleotide sequence of SEQ ID NO: 2.

14. An industrial mixture suitable for degrading cellulose, such mixture comprising the thermostable AviIII polypeptide of claim 1.

15. (Amended) The industrial mixture of claim 14 further defined as comprising a detergent.

28. (Amended) An isolated polypeptide molecule comprising:

a) a polypeptide sequence of SEQ ID NO: 3;

- b) a polypeptide sequence of SEQ ID NO: 4;
- c) a polypeptide sequence of SEQ ID NO: 5;
- d) a polypeptide sequence of SEQ ID NO: 1; [or]
- e) a polypeptide sequence of SEQ ID NO: 3; SEQ ID NO:4; and SEQ ID NO: 5;

or

f) a sequence having at least about 70% sequence identity with the polypeptide sequence of a), b), c), d), or e).

29. (Amended) The polypeptide molecule of claim 28, having at least about 90% sequence identity with the polypeptide sequence of a), b), c), d), e), or f).

30. A fusion protein comprising the polypeptide of claim 28 and a heterologous peptide.

31. The fusion protein of claim 30, wherein the heterologous peptide is a substrate targeting moiety.

32. The fusion protein of claim 30, wherein the heterologous peptide is a peptide tag.

33. (Amended) The fusion protein of claim 32, wherein the peptide tag is 6-His, thioredoxin, hemagglutinin, glutathione S-transferase [GST], or OmpA signal sequence tag.

34. The fusion protein of claim 30, wherein the heterologous peptide is an agent that promotes polypeptide oligomerization.

35. The fusion protein of claim 34, wherein the agent is a leucine zipper.

36. A cellulase-substrate complex comprising the isolated polypeptide molecule of claim 28 bound to cellulose.

**REMARKS**

Claims 1-15 and 28-36 are pending in the application. Claims 1, 3-13, 15, 28, 29 and 33 have been amended.

**Specification**

The objections to the Specification on page 2 of the Office Action dated August 1, 2002 are noted. As to the objections to the citation format used extensively in this application, for example, where citations are made by way of citation sentences on page 3 at lines 14-15, Applicants' attorney respectfully submits that this is a proper and commonly accepted citation format. The citation is understood to support the remarks preceding the citation. Exhibit 1 is a case, *Genentech Inc. v. Amgen Inc.*, 62 U.S.P.Q.2d 1640 (Fed. Cir. 2002), that shows the Court of Appeals for the Federal Circuit uses this citation format. In Exhibit 1, the last two lines from the bottom of page 3 show this manner of citation, as does the carryover paragraph on page 13 in numerous instances. Amendment is not required, as use of this manner of citation is neither a defect nor an error.

The requirement to identify the glycoside hydrolase domain and the carbohydrate binding domain of "cellulases" is not understood insofar as the requirement is to identify these features as conserved sequences for all cellulases, generally, by way of amendment to the specification. Tables 3, 4 and 5, for example, identify these sequences for AviIII, particularly, with regard to the GH74 family catalytic domain. Is the Examiner requesting amendment to the specification to document conserved sequences for all cellulases, or the GH74 family as shown?

Page 1 has been amended to clarify that the citations to Wiselogel and Bergeron are actually part of the *Handbook on Bioethanol*.

A replacement drawing with Fig. 2 thereon is filed herewith.

Replacement page 6 to the Specification amends Table 4 to recite --kDa--, as opposed to "KB."

Amended claim 1 corrects the spelling of "AviIII."

#### **Claims – 35 U.S.C. §101**

Claims 12 and 13 are rejected under 35 U.S.C. §101 as being directed towards nonstatutory subject matter. This objection is overcome, as suggested by the Examiner, by reciting that the peptides are "isolated."

#### **Claims – 35 U.S.C. §112, Second Paragraph**

Claims 4, 5, 7, 8, 9-11, 13, and 33 are rejected under 35 U.S.C. §112, second paragraph, and have each been amended to overcome the objections to these claims of pages 3-5 of the Office Action dated August 1, 2002.

Claims 4, 5, 7, and 8 are objected to as being multiple dependant claims that improperly depend from other multiple dependant claims, and because claim 4 depends from itself. This objection is overcome by amending each of claims 4, 5, 7 and 8 to delete reference to claim 4. Reference to claim 3 was intended.

Claims 9-11 are objected to for indefiniteness. The objection to claim 9 has been overcome by amending claim 9 to recite --polypeptides represented by—the SEQ Ids, as recommended by the Examiner. Claims 10 and 11 have been amended to recite that the polypeptide is encoded by the nucleic acid sequence. The objection to claim 13 is overcome in the same manner.

Claim 1 has been amended to recite the meaning of “GH,” which at first appearance is glycosyl hydrolase, and to recite the article “a” carbohydrate binding domain. Claim 33 is amended to recite glutathione S-transferase in place of “GST.”

Claims 10, 11, 28 and 29 have been amended to recite at least XX% sequence identity.

**Claims – 35 U.S.C. §112, First Paragraph**

Claims 1-9, 14, 15, 28-36 and 43 stand rejected under 35 U.S.C. §112, first paragraph, for being broader than the enablement provided by the written description. This rejection is postulated because the disclosure is limited to the specific sequences disclosed, but the claims address any thermostable AviIII composition.

The standard for making a rejection based on 35 U.S.C. § 112, first paragraph, is articulated in *In re Wands*, 858 F.2d 731 (Fed. Cir. 1988) (see also MPEP § 2164.01 and 2164.04). Initially, the Office must accept the objective truth of statements made in the specification. If such statements are to be called into question, the Office is burdened with providing evidence or convincing argument why those of skill in the art would doubt the statements (*In re Marzocchi*, 439 F.2d 220, 169 USPQ 367 (CCPA 1971)). Applicant asserts that this burden has not been met.

In *In re Wands*, wherein the issue was the predictability of being able to make a particular monoclonal antibody, the court states that “[e]nablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. The key word is ‘undue,’ not experimentation.” The court further states that “[t]he determination of what constitutes undue experimentation in a given case requires the

application of a standard of reasonableness, having due regard for the nature of the invention and the state of the art. The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.” In the present case, Applicants have provided sufficient guidance to enable the broader claims in light of the art and the level of skill, for example, in Example 2 and Table 3 on pages 32-33 of the Specification. Withdrawal of the rejection is respectfully requested.

As to claims 1, 14, and 15, the Examiner further asserts that knowledge and guidance are required to determine which amino acids, if any, are tolerant of modification, as well as those which are conserved and presumably not subject to modification. Applicants respectfully traverse the rejection because such information is provided, for example, on pages 32-33 of the Specification in Example 2 and Table 3, which provide those skilled in the art with sufficient information to make these determinations by observing the conserved sequences among the GH74 family. The remaining objections as to claims 2-5, 6-9, 28-36, and 43 are postulated for identical reasons, and Applicants similarly traverse these objections.

Applicants traverse the statements on page 7 of the Office Action dated August 1, 2002, because the specification does support the broad scope of claims 2-9. The broader scope is enabled, for example, by the showing of conserved regions in Example 2 and Table 3 on pages 32-33 of the specification. The scope of the claims does bear a reasonable correlation to the scope of enablement.



Page 8 of the Office Action dated august 1, 2002 further rejects claims 1-9, 14 and 15 for the reason that only a single representative species of the claimed genus is disclosed. "Compliance with the enablement requirement of 35 U.S.C. § 112, first paragraph, does not turn on whether an example is disclosed." M.P.E.P. § 2164.02. In fact, "[t]he specification need not contain an example if the invention is otherwise disclosed in such manner that one skilled in the art will be able to practice it without an undue amount of experimentation." See *In re Borkowski*, 422 F.2d 904, 908 (CCPA 1970). In the present case, Applicants have disclosed a working embodiment and taught others how to create like-functional compositions, for example, by identifying and comparing highly conserved sequences in Example 2 and Table 3. We submit that the Specification in light of the art and the level of skill refutes these hypotheses rejecting the claims, and that no *prima facie* case for lack of enablement has been established.

Claims 28-36 and 43 are further rejected because the claims are directed to a genus of sequence homology, e.g., 70% identity with SEQ ID No. 1, but none of the homologous sequences are specifically disclosed. It is well within the ordinary skill in the art to create sequence changes, e.g., by site-directed mutagenesis, sequence truncation, or those in the nature of a fusion protein. *In re Buchner*, 929 F.2d 660, 661 (Fed. Cir. 1991), the court stated that "[a] patent need not teach, and preferably omits, what is well known in the art." It was unnecessary for applicant to disclose in the instant specification further details of how to alter sequences when techniques for doing so are widely known in the art.

### **Claims – 35 U.S.C. §102**

Claims 1-13, 28, 29 and 43 stand rejected under 35 U.S.C. §102(b) as being anticipated by United States Patent No. 5,110,735 issued to Tucker et al. (Tucker 1992), or United States Patent No. 5,366,884 issued to Adney et al (Adney 1994), as evidenced by Klasrskov (1997), Harrison et al. (1998), and United States Patent No 5,712,142 issued to Adney et al. (Adney 1998).

The rejection postulates an unwarranted assumption that the cellulases are identical, for example, because the Abstract of Tucker 1992 and Example 7 of Adney 1994 each report an enzyme having a molecular weight of from 156,000 to 203,400 daltons, which possesses C1 and Cx types of enzymatic activity. The Examiner observes that the deduced molecular weight of the claimed AviIII cellulase based upon SEQ ID NO. 1 is approximately 105 kDa, which is smaller than the size of the high molecular weight cellulase disclosed by Tucker 1992 and Adney 1994, and larger than the low molecular weight cellulase disclosed by Tucker 1992 and Adney 1994. The remaining documents are used to show glycosylation of cellulases. An allegation is raised that the claimed cellulase is the same as the high molecular weight cellulase reported by Tucker 1992 and Adney 1994, with glycosylation accounting for the weight discrepancy. We respectfully traverse that allegation.

AviIII cannot be the same high molecular weight cellulase taught by Tucker 1992 and Adney 1994. *Acidotherrnus*, and most other bacteria that secrete proteins, do not glycosylate them. For example, we attach Exhibit 2, which is a current commercial publication from Sigma Aldrich taken from:

[http://www.sigmaaldrich.com/Brands/Fluka\\_Riedel\\_Home/Organic\\_Synthetic/Enzymes/Bioanalytical\\_Chemistry/Glycosidases.html](http://www.sigmaaldrich.com/Brands/Fluka_Riedel_Home/Organic_Synthetic/Enzymes/Bioanalytical_Chemistry/Glycosidases.html)

Exhibit 2 specifically states a well-know problem in the art, and follows this principle with a listing of commercial products for accomplishing glycosylation of bacteria-expressed proteins:

Glycosylation of biomolecules, especially proteins, is of major importance for their biological activity. Thus investigation of glycosylation patterns is crucial in life sciences. As bacteria lack ability to glycosylate proteins, glycosylation is a major issue for use of recombinant proteins. Selective hydrolysis by glycosidases is applied for characterization of glycoproteins.

Glycosylation of secreted extracellular enzymes is common in eukaryotes, but it is well-known that bacteria use other mechanisms to transport proteins outside the cell wall. Bacterial enzymes, such as cellulases produced by *Acidothermus*, are not glycosylated. Therefore, the 105 kDa Avicellase of this application cannot be the endoglucanase(s) taught by Tucker 1992 or Adney 1994 on the basis of molecular weight alone.

A Declaration Under Rule 132 is attached. The declaration is provided to present the Examiner with additional facts that deserve consideration and are relevant to the §102 rejection, as well as the §103 rejection below. The Declaration contains additional statements regarding the inability of *Acidothermus* to glycosylate cellulases. The Declaration also provides additional information regarding the serendipitous discovery of AviIII, which was discovered as a xyloglucanase gene attached to an endoglucanase fragment when screening for endoglucanases. AviIII-derived cellulases cannot be the high molecular weight composition identified as an endoglucanase in Tucker 1992 and Adney 1994, as indicated by the xyloglucanase versus endoglucanase distinction.

#### **Claims – 35 U.S.C. §103**

Claims 1-13, 28, and 29 stand rejected under 35 U.S.C. §103(a), as being unpatentable over Mohaghegi et al. 1986 in view of 'Berghem et al. 1976 and Katz et al.

1968. Mohaghegi et al. 1986 is said to show the isolation of *Acidothermus cellulolyticus*, but not the isolation of cellulase therefrom. Berghem et al. 1976 is used to show the isolation of cellulase from *Trichoderma viride*. Katz et al. supposedly shows motivation to combine, since it is desirable to generate alternative cellulases capable of commercial scale processing. This supposed motivation is nothing more than generalized finger-pointing towards a desirability of isolating additional cellulases, and it utterly fails to address identification of the specific cellulase now being claimed. Katz et al. 1968 is nothing more than evidence of long felt need because the general directive to isolate additional cellulases for large scale commercial use was unsatisfied at the time of the present invention—and it has existed that way since 1968.

There is no nexus between *Acidothermus cellulolyticus* and *Trichoderma viride*. Nothing can be deduced from the isolation of cellulase from *Trichoderma viride* which would lead those skilled in the art to deduce that the GH74 family cellulase with features now being claimed could be isolated from *Acidothermus cellulolyticus*. This is particularly true when other researchers (Tucker 1992, Adney 1994) were engaged in *Acidothermus cellulolyticus* research and merely discovered different endoglucanases reporting activity on CMC, i.e., other activity to identify cellulases from *Acidothermus* has not developed the composition now being claimed.

As shown in the accompanying Declaration Under Rule 132, Tucker 1992 and Adney 1994 fail to teach or suggest the AviIII-derived cellulases. The AviIII cellulases are not glycosylated by *Acidothermus* and are xyloglucanases. These are not the endoglucanases taught by Tucker 1992 and Adney 1994. There was no reason to expect, at the time of the invention, that additional *Acidothermus* work would succeed in

identifying another cellulase, in this case a xyloglucanase, even if Katz et al. does state that it is desirable to isolate additional cellulases.

Claim 14 stands rejected under 35 U.S.C. §103(a) as being unpatentable over Tucker et al. 1992 or Adney et al. 1994 in view of Vollmond et al. 1999 and Katz et al. 1968. Tucker 1992 and/or Adney 1994 are applied as above. Vollmond et al. 1999 is used to show use of an acidophillic cellulase in industrial solutions for degrading fabrics, and Katz et al. 1968 is used to suggest the finding of alternative cellulases. The inapplicability of Tucker et al. 1992 and Adney et al. 1994 is demonstrated above. Since the combination documents fails to teach or suggest the claimed composition, the rejection cannot stand. Withdrawal of the rejection is respectfully requested.

In fact, claims 14, 30-33, 36, 43 are all rejected under 35 U.S.C. §103(a) over various combinations, all of which rely exclusively upon Tucker et al. 1992 or Adney et al. 1994 to show the basic AviIII composition of claim 1. That reliance is misplaced because the molecular weights are different, and there is a xyloglucanase versus endoglucanase distinction. Thus, any combination of these references fails to teach the claimed Avicellulase. Furthermore, motivation to combine or modify references is lacking where there is no teaching or suggestion to arrive at the composition that is specifically claimed.

Applicants' attorney respectfully solicits a Notice of Allowance in this application. The Commissioner is authorized to charge any additionally required fees to deposit account 14-0460. Should the Examiner have any questions, comments, or suggestions that would expedite the prosecution of the present case to allowance, Applicants' representative, Paul White, earnestly requests a telephone call at (303) 384-7575.

Respectfully submitted

A handwritten signature in black ink, appearing to read 'Dan Cleveland, Jr.', with a stylized, cursive script.

Dan Cleveland, Jr. Reg. No. 36,106  
Lathrop & Gage, L.C.  
4845 Pearl East Cir, Suite 300  
Boulder, CO 80301  
(720) 931-3012  
(720) 931 3001 (Fax)

Table 4. Nucleotide and polypeptide segments.

AvIII Segment	base BEGIN	base END	Length, bp	aa BEGIN No.	aa	aa END No.	aa	Length, aa	SEQ ID No. (amino acid)	SEQ ID No. (nucleotide)
Total length	1	about 3000	about 3 kb	1	M	about 1000	X*	about 1 [kb]kDa	1	2
Signal (potential)	1	108	108	1	M	36	A	36		
CD (GH74)	109	2328	2220	37	A	776	G	740	3	
CBD_III (partial)	2575	about 3000	about 0.5 kb	859	V	about 1000	X*	about 154	4	
CBD_III (partial)	2575	2838	264	859	V	946	Q	88	5	

**FULL TEXT OF CASES (USPQ2D)**  
All Other Cases

**Genentech Inc. v. Amgen Inc., 62 USPQ2d 1640 (CA FC  
2002)**

**62 USPQ2D 1640**  
**Genentech Inc. v. Amgen Inc.**

**U.S. Court of Appeals Federal Circuit**

**No. 01-1098**  
**Decided April 29, 2002**

**Headnotes**

**PATENTS**

**[1] Infringement — Construction of claims (§120.03)**

**Infringement — Literal infringement (§120.05)**

**Patent construction — Claims — Defining terms (§125.1305)**

**Improper claim construction underlies summary judgment that defendant's protein product did not literally infringe patents directed to method and cloning vehicle for expression of gene into protein, since term "ribosome binding site," which refers to constituent of expression control region of cloning vehicle, is properly construed in accordance with its function to require only Shine-Dalgarno sequence and start codon that are capable of binding ribosome and initiating translation of mRNA into protein, since linker DNA sequence between S-D sequence**



and start codon thus is not required functional element of “ribosome binding site,” and since claimed “control region” may be constructed from multiple operons, or synthesized, and need not

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be derived from single operon as long as region's sequence corresponds to sequence found in single operon; on remand, district court must determine whether sequences in accused cloning vehicle that are identical to sequences of plaintiff's cloning vehicle are sufficient for initiation of translation.

[2]

**Infringement — In general (§120.01)**

## **JUDICIAL PRACTICE AND PROCEDURE**

**Procedure — Discovery — In general (§410.4001)**

Federal district court did not abuse its discretion by denying patent infringement plaintiff's motion for discovery of nucleotide sequence of entire “control element” within accused cloning vehicle, and method of making same, which plaintiff sought for purpose of determining whether control region falls within scope of claim limitation requiring sequence of control element to correspond to single operon, since plaintiff need only compare sequence of “ribosome binding site” portion of control region to determine whether its claimed control region is infringed, and since RBS sequences are already available to plaintiff.

## **PATENTS**

**[3] Infringement — Doctrine of equivalents — In general (§120.0701)**

Federal district court did not clearly err in barring plaintiff from proceeding on theory of infringement under doctrine of equivalents, since plaintiff did not include theory in claim chart as required by local rule, and since plaintiff could not have believed in good faith that claim chart did not constitute its final commitment to theories of infringement that it could pursue; plaintiff's argument that defendant had notice of its doctrine of equivalents theory prior to claim construction hearing is unavailing, since policy underlying rule governing amendment of claim charts is conservative, and since plaintiff has not shown legal or factual error in district court's ruling.

## Particular Patents

**Particular patents — Chemical — Genetic expression**  
**4,704,362, Itakura and Riggs, recombinant cloning vehicle for microbial polypeptide expression, summary judgment of noninfringement vacated.**

**5,221,619, Itakura and Riggs, method and means for microbial polypeptide expression, summary judgment of noninfringement vacated.**

**5,583,013, Itakura and Riggs, method and means for microbial polypeptide expression, summary judgment of noninfringement vacated.**

### **Case History and Disposition**

**Appeal from the U.S. District Court for the Northern District of California, Alsup, J.**

**Action by Genentech Inc. against Amgen Inc. for patent infringement. Federal district court granted summary judgment of no literal infringement, denied plaintiff's motion for discovery, and barred plaintiff from asserting infringement under doctrine of equivalents pursuant to local rule. Summary judgment vacated and remanded; denial of discovery motion and enforcement of local rule affirmed.**

### **Attorneys:**

**Leora Ben-Ami and John E. Kidd, of Clifford, Chance, Rogers & Wells, New York, N.Y.; John S. Skilton, M. Patricia Thayer, and Elizabeth A. Brown, of Heller, Ehrman, White & McAuliffe, San Francisco, Calif., for plaintiff-appellant.**

**Lloyd R. Day Jr., Jackie N. Nakamura, and Jeffrey K. Lee, of Day, Casebeer, Madrid & Batchelder, Cupertino, Calif.; William G. Gaede III, Andrew A. Kumamoto, Madison C. Jellins, and Monica K. Hoppe, of Cooley Godward, San Francisco, for defendant-appellee.**

### **Judge:**

**Before Michel, Rader, and Schall, circuit judges.**

## Opinion Text

### **Opinion By:**

**Rader, J.**

On summary judgment, the U.S. District Court for the Northern District of California determined that Amgen, Inc. (Amgen) did not literally infringe Genentech, Inc.'s (Genentech's) U.S. Patent Nos. 4,704,362 (the '362 patent), 5,221,619 (the '619 patent), and 5,583,013 (the '013 patent). *Genentech, Inc. v. Amgen, Inc.*, No. C 96-03752 WHA, slip op. at 1-2 (N.D. Cal. Oct. 12, 2000) (*Amended Summary Judgment Order*). The

district court also barred Genentech from proceeding on a theory of infringement under the doctrine of

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equivalents. *Id.* Because the district court did not abuse its discretion, this court affirms the district court's decision to preclude Genentech from asserting infringement under the doctrine of equivalents. The district court, however, relied on an erroneous claim construction in granting Amgen's motion for summary judgment. Accordingly, this court vacates and remands for a determination of infringement under this court's revised claim construction.

I.

Genentech owns the '362, '619 and '013 patents. These patents claim methods and cloning vehicles for the introduction and expression of genetic information, i.e., deoxyribonucleic acid (DNA) or genes, in unicellular organisms that do not naturally contain or express that genetic information. The patents thus enable introduction of a DNA sequence, such as a synthetic gene that expresses a usable protein, into cells via a cloning vehicle. The cells then express this sequence through the endogenous protein-making machinery of the cell. The inventions thus enable harvesting valuable proteins from single cell "factories." Inside a bacterial cell, the expression of a gene into a protein involves a two-step process. First, the cell transcribes the DNA sequence into messenger ribonucleic acid (mRNA) by an enzyme called RNA polymerase. The RNA polymerase then binds to a specific sequence within the DNA known as a "promoter" upstream from the DNA sequence encoding the usable protein. To control transcription, and therefore protein expression, the DNA sequence upstream from the usable gene also contains a site called an "operator." The operator controls transcription by binding a protein known as a "repressor." When the repressor binds to the operator, the repressor prevents the RNA polymerase from binding to the promoter, and therefore blocks transcription. After transcribing a DNA sequence into mRNA, the cell engages in the second step, translation of the mRNA into protein. Specifically, a ribosome, which is a cellular structure involved in converting mRNA to polypeptides, binds to an upstream portion of the mRNA sequence known as the "ribosome binding site." This binding triggers translation of the mRNA into a linear chain of amino acids — a protein. The three patents at issue, which contain nearly identical specifications, describe the use of a recombinant cloning vehicle (e.g., a plasmid, comprising a circular piece of non-chromosomal double-stranded DNA) to transform a unicellular organism host such as the bacterium *E. coli* to enable that organism to make large amounts of a protein that it would not otherwise produce. The first claim in all three patents sets forth the invention:

'362 claim 1:

A recombinant DNA cloning vehicle suited for transformation of a microbial host comprising

- (a) a *homologous control region* which regulates expression of a structural gene and
- (b) a DNA insert comprising ... in that the DNA insert is ... and the host transformed thereby is capable of expressing ... under the control of the said control region and in recoverable form.

'619 claim 1:

A process for the production of a polypeptide comprising a preselected functional

mammalian polypeptide or polypeptide intermediate therefor in a microbial cell culture, said process comprising

(i) effecting expression of said polypeptide in a microorganism transformed with a replicable cloning vehicle comprising DNA encoding said polypeptide which DNA is *under the control of an expression control region homologous to said microorganism*; and

(ii) recovering the polypeptide from said cell culture.

'013 claim 1:

A process for the production of a polypeptide comprising ...effecting expression of said polypeptide in a microorganism transformed with a replicable cloning vehicle comprising DNA encoding said polypeptide which DNA is *under the operative control of an expression control region functional in E. coli comprising operatively linked promoter, operator and ribosome binding site DNA*. (Emphasis added.)

In its 17 May 1999 claim construction order, the district court (Judge Smith) construed

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the relevant terms in the claims. *Genentech, Inc. v. Amgen, Inc.*, No. C96-3752 FMS, slip op. at 1 (N.D. Cal. May 17, 1999) (*Order*). The district court determined that the “control region,” which regulates gene expression, contains at least three control elements: a promoter, an operator, and a ribosome binding site. *Id.* at 24. As construed by the district court, the “control region” in all three patents must be taken from a single “operon”(i.e., DNA comprising a control region and the gene whose expression is regulated by that control region).<sup>1</sup> *Id.* at 20-23. Variation from the native control region of the untransformed host is permissible as long as the control region remains operable, i.e., the control region need not be intact. *Id.* at 20.

The court construed “homologous” in the '362 and '619 patents to mean that the control region DNA sequence is taken from, and ordinarily is endogenous to, the host DNA in its untransformed state. *Id.* at 12. To be endogenous to the host, DNA must be either part of the chromosomal host DNA, part of the plasmid DNA native to the host, or part of the chromosomal or plasmid DNA native to a “bacteriophage” (i.e., a bacterial virus from which one may derive a plasmid) ordinarily found in the bacterial host cell. *Id.* A control region is not homologous if it includes any alterations in the endogenous sequence, with the single exception of a promoter mutation described in the patent, namely, deletion of the catabolite activator protein (CAP) binding site. *Id.* at 10-12; '362 patent, col. 2, ll. 31-35.2

The district court construed the term “functional in *E. coli*” to mean that the control region performs in *E. coli*. Although operative in *E. coli*, the term does not mean that the control region must be homologous to *E. coli*. *Id.* at 15. The district court also interpreted the term “operatively linked” to mean that the promoter, operator, and ribosome binding site (P, O, and RBS) are sufficiently connected to direct and regulate expression. *Id.* at 34.

This term does not require that the P, O, and RBS appear in any particular order.

As to the term “ribosome binding site,” the district court originally construed it to mean “a DNA sequence that is an irreducible constituent of the expression control region that, when transcribed into mRNA, is bound by the ribosome, and is thus required for the initiation of translation.” *Id.* at 50. After Judge Smith issued the 17 May 1999 claim construction order, the case was transferred to Judge Alsup. In its 12 October 2000

amended order granting Amgen's motion for partial summary judgment, the district court elaborated: "Judge Smith's requirement that the ribosome binding site be 'bound' by the ribosome is best understood to mean that the ribosome binding site consists of the entire sequence encompassed (or bound) by the two RNA sites with which the ribosome interacts to initiate translation." *Amended Summary Judgment Order* at 13.

Amgen makes and sells Neupogen®, a recombinant methionyl human granulocyte colony stimulating factor (met-hGCSF) protein. This protein accelerates the replication of human white blood cells. Amgen employs *E. coli* to produce Neupogen® with a recombinant plasmid containing the gene for met-hGCSF. *Original Summary Judgment Order*, at 2. The regulatory region (P, O, and RBS) of Amgen's plasmid is derived in part from the bacteriophage lambda. The first 72 base pairs of the Amgen regulatory region are identical to the first 72 base pairs of the endogenous lambda regulatory region. These 72 base-pairs encompass the promoter and operator in both regulatory regions. *Id.* at 4. As noted by the district court, the RBS includes, at minimum, a Shine-Dalgarno (S-D) sequence (a conserved sequence of five nucleotides in this case) and a start codon, ATG (the first three nucleotides translated into an amino acid). *Id.* The ribosome binds the mRNA at these two sequences. The S-D sequence and the start codon are separated by "linker" base-pairs (usually about 10 base-pairs) that do not actually bind the ribosome. The district court found, however, that these linker base-pairs,

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like the S-D sequence and the start codon, are necessary for the initiation of translation. *Id.*

Importantly, the base-pairs linking the S-D sequence and the start codon in Amgen's plasmid are different in number and identity from the linker base-pairs in the endogenous lambda sequence:

bacteriophage lambda:

AGGAGAATCCAGATG

Amgen's plasmid:

AGGAGGTAATAAATAATG *Id.* at 5.

Because the district court determined that a "control region" (P, O, and RBS) must come from a single operon, and that a "ribosome binding site" encompasses the S-D sequence, the start site, as well as the linker base-pairs, the district court found no literal infringement. Specifically, although Amgen's P and O came from the lambda operon, a comparison of the sequences shows that Amgen's RBS (with its alternative linker sequence) did not. In granting summary judgment of no literal infringement, the district court also denied further discovery. According to the district court, Amgen's process for deriving its RBS was irrelevant because Amgen's plasmid RBS sequence did not match the RBS of lambda. *Id.* at 12.

Applying its local rules, the district court concluded that Genentech did not allege infringement under the doctrine of equivalents (DOE) in its complaint or in its Civil Local Rule 16-9 Claim Chart. *Id.* at 7. In the Northern District of California, under Civil Local Rule 16-9(a)(3), a claim chart must state "whether such infringement is claimed to be literal or under the doctrine of equivalents." Civ. L.R. 16-9(a)(3). The district court rejected the proposition that Genentech's failure to include in its chart a claim of infringement by equivalents was simply excusable oversight. *Original Summary*

*Judgment Order* at 8.

Genentech appeals the district court's summary judgment of no literal infringement and its enforcement of the local rule to bar a theory of infringement under the doctrine of equivalents. This court has jurisdiction under 28 U.S.C. §1295(a)(1) (1994).

## II.

This court reviews without deference a district court's grant of summary judgment and draws all justifiable inferences in favor of the nonmovant. *Anderson v. Liberty Lobby, Inc.*, 477 U.S. 242, 255 (1986); *Johns Hopkins Univ. v. Cellpro, Inc.*, 152 F.3d 1342, 1353, 47 USPQ2d 1705, 1713(Fed. Cir. 1998). This court also reviews claim construction without deference. *Cybor Corp. v. FAS Techs., Inc.*, 138 F.3d 1448, 1456, 46 USPQ2d 1169, 1174(Fed. Cir. 1998) (en banc).

On procedural issues not unique to this court's exclusive jurisdiction, this court applies the procedural law of the regional circuit, in this case the United States Court of Appeals for the Ninth Circuit. *Vivid Techs., Inc. v. Am. Sci. & Eng'g, Inc.*, 200 F.3d 795, 807, 53 USPQ2d 1289, 1297(Fed. Cir. 1999). The Ninth Circuit reviews a district court's denial of a motion under Fed. R. Civ. P. 56(f) for an abuse of discretion. *Garrett v. City of San Francisco*, 818 F.2d 1515, 1518 (9th Cir. 1987). The Ninth Circuit reviews a district court's application of local rules and decision to enforce a pretrial order's limits on theories of liability for an abuse of discretion. *Acorn v. City of Phoenix*, 798 F.2d 1260, 1272 (9th Cir. 1986); *United States v. Warren*, 601 F.2d 471, 474 (9th Cir. 1979). The Ninth Circuit reviews a district court's evidentiary rulings for an abuse of discretion. *United States v. Meyers*, 847 F.2d 1408, 1411 (9th Cir. 1988).

## III.

Genentech argues on appeal that the term “ribosome binding site” in claim 1 of the '013 patent encompasses only the S-D sequence and the start site because they are the only sequences that bind the ribosome to initiate translation. Thus, according to Genentech, the RBS, and consequently the “control region” of claims 1 in the '362 and '619 patents, do not include the “non-functional” linking nucleotides that do not directly bind the ribosome. This assertion is important to Genentech's assertion of literal infringement because Amgen's plasmid regulatory region (P, O, and RBS) differs from the endogenous lambda regulatory region only in the RBS linker sequence. If “ribosome binding site” includes only the S-D sequence and the start codon, then Amgen's regulatory region is homologous to the lambda regulatory region, and is potentially derived from a single operon, as required under the district court's claim construction

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of “homologous” and “control region.”

The specification does not explicitly define “ribosome binding site.” Claim 1 of the '013 patent uses the term without explanation. The specification mentions the term once when referring to the regulatory element derived from lambda DNA. '013 patent, col. 9, ll. 2-8. Figure 5A in the '362 and '013 patents refers to the “Ribosome Protected RNA,” which shows the area where the ribosome protects the RNA from degradation. This “Ribosome Protected RNA” does not necessarily equate to the RBS, however, because the ribosome may protect more RNA than is actually involved in transcription initiation, such as nucleotides close to the RBS.

The prosecution history sheds some light on the meaning of RBS. As stated in an amendment submitted by the patentee to the PTO on December 16, 1986:

Shine and Dalgarno showed that *nucleotides within a ribosome binding site can form base pairs with complementary nucleotides within 16S ribosomal RNA*, thereby suggesting an explanation for *how* ribosome binding sites participate in the initiation of translation. See for example the following publications []: [citations omitted]. These early papers, the latest of which was published almost two years before applicants' filing date, certainly demonstrate that by the time of the filing of the present application [the '362 patent, filed Nov. 5, 1979] the art was well aware of the fact that *a S-D sequence was one of the necessary elements of the control region that directs the expression of structural genes*.

\* \* \* \*

Both Figures [5A and 5B] clearly show the ... AGGA ...sequence, 8-11 nucleotides before the ATG start codon, which is the Shine-Dalgarno sequence of the *lac* control region. *It is one of the "control elements" of the lac operon* and one of the "key portion(s)" of the plasmids shown.

\* \* \* \*

The quoted section [from the section entitled "Plasmid Construction Generally"] unequivocally demonstrates the presence in the specification of an explicit teaching of *the ribosome binding site, which includes the S-D sequence*, as a necessary element in regulating expression.

Amendment submitted to U.S. Patent and Trademark Office, U.S. Application Serial No. 06/090,979, at 11, 14, 15 (Dec. 16, 1986) (*Amendment*) (emphasis added).

Thus, in the prosecution history, the inventors defined the S-D sequence as a necessary element of the ribosome binding site. *Id.* at 11, 14. Because the ribosome binding site "includes the S-D sequence," however, the ribosome binding site clearly includes something else. The prosecution history suggests that the ATG start site also is part of the ribosome binding site. As stated by the patentee during prosecution:

[T]he thrust of [a prior art] patent is the introduction of an alleged "hybrid" ribosome binding site in which the ATG codon is brought in with the heterolog[o]us DNA rather than being present in the homologous control region. Since "ATG" is "ATG" regardless of its origins, this is a distinction without a difference.

*Id.* at 18. In addition to the S-D sequence, therefore, the ribosome binding site also must include at least the start codon. The start codon binds directly to the ribosome (via complementary nucleotides within the 16S ribosome RNA), and is a "necessary element[]" of the control region that directs the expression of structural genes." *Id.* at 11.

The patent and its prosecution history, however, do not suggest whether the ribosome binding site also encompasses the linker sequence. Moreover, extrinsic evidence, such as testimony from Amgen's expert, Dr. Alexander Johnson, and Genentech's expert, Dr. Jeffrey Ravetch, does not speak directly to this issue. In his declaration submitted to the district court, Dr. Johnson states:

In bacteria, ribosomes differentiate by initially binding with specific sequences, up to six nucleotides long, located upstream from the initiation codon, and then through a complex reaction, interact with the start codon to initiate translation. Collectively, these regions of nucleotides are known as the "ribosome binding site" and include all sequences necessary to initiate translation.

Johnson Decl., ¶ 32. Although Dr. Johnson appears to suggest that the S-D sequence and the start codon “are known as the `ribosome binding

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site,” he also asserts that the RBS “include[s] all sequences necessary to initiate translation.” Thus, Dr. Johnson's declaration leaves open the question of whether the linker sequences might be among those necessary to initiate translation. Dr. Ravetch, on the other hand, expressly states in a declaration: “There are two components which make up the ribosome binding site: the Shine Dalgarno sequence and the initiation codon ATG. ... A ribosome binding site is a sequence of DNA that when transcribed into RNA is capable of binding a ribosome and initiating translation.” Ravetch Decl., ¶ 9.

[1] In sum, the entire record — both intrinsic and extrinsic evidence of claim meaning — does not establish that the linker DNA sequence is a required functional element of the RBS. To the contrary, the record adequately supports the district court's original claim construction of “RBS” — “a DNA sequence that is an irreducible constituent of the expression control region that, when transcribed into mRNA, is bound by the ribosome, and is thus required for the initiation of translation.” *Order* at 50. The record shows that Judge Alsup's later interpretation of Judge Smith's claim construction order (requiring the RBS to include the linker DNA sequence between the S-D sequence and the start codon) is incorrect. *Amended Summary Judgment Order* at 13.

While Judge Smith's construction correctly specifies that the ribosome binding site, when transcribed into mRNA, is bound by the ribosome and is required for translation, it may unintentionally suggest that a sequence that is necessary but not sufficient for the initiation of translation satisfies this claim limitation. Such an interpretation would include within the ambit of a ribosome binding site a single base pair that is bound by the ribosome and is necessary for the initiation of translation, irrespective of whether that base pair alone (along with the promoter and operator) can perform the desired function of initiating translation. Judge Smith doubtlessly did not intend such a result. Therefore this court clarifies Judge Smith's interpretation. The claim term “ribosome binding site” is properly construed according to its function of being bound by the ribosome and initiating translation, irrespective of whether, as a general proposition outside of this context, the linker DNA between the Shine-Dalgarno site and ATG start codon is included within the scope of the claim term. This court therefore adopts Judge Smith's original construction of “ribosome binding site” with slight modification and interprets it to mean a “DNA sequence that is an irreducible constituent of the expression control region that, when transcribed into mRNA, is bound by the ribosome and is thus necessary and sufficient to initiate translation.”

With respect to the term “control region,” which appears in the representative claims of all three asserted patents, the district court originally propounded the following construction:

“a piece of DNA, containing at least a promoter, an operator, and a ribosome binding site, that is the part of the recombinant DNA cloning vehicle that directs and regulates expression of the structural gene. The control region must be taken from a single operon; it may not be constructed from control elements derived from various operons.”



*Id.* at 24. Judge Smith further found that “DNA [comprising the control region] may be ‘taken from’ the listed sources: it may be physically obtained, cloned, partially chemically synthesized or totally chemically synthesized.” *Id.* at 37-38.

Nevertheless, in his summary judgment ruling, Judge Alsup interpreted Judge Smith's claim construction to include a limitation on the method of obtaining the control region. In confirming that the definition of “ribosome binding site” necessarily includes the linker DNA, Judge Alsup stated that “were the term ‘ribosome binding site’ reduced to the Shine-Dalgarno sequence and the start codon ATG, it would be impossible to determine whether a ribosome binding site was derived from the same operon as the promoter and the operator, a requirement of the term ‘control region.’” *Amended Summary Judgment Order* at 14. Judge Alsup assumed that the control region must possess control elements that correspond to one and only one operon. Hence, Judge Alsup concluded that Amgen's ribosome binding site DNA was not physically “taken from” the bacteriophage lambda. *Id.*

Genentech asserts that by this language, Judge Alsup misapprehended the meaning of the claim term “control region” by requiring (1) that the method used to construct the control region use only one operon, and (2) that

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to prove this single operon source requirement was satisfied, the sequence of each control element must correspond to a control element found in one, *and only one*, operon.<sup>3</sup>

This court agrees with Genentech on this point.<sup>4</sup> The district court did not appreciate the distinction between the content (sequence) of the control region and the method by which one constructs the control region. Genentech does not argue that the patent supports an interpretation of “control region” that includes so-called hybrid control regions in which the operator, promoter, and ribosome binding site sequences do not correspond to the three control elements' sequences found in a single operon. Rather, it argues that as long as the sequence of the control elements correspond to the sequence of the control elements found in a single operon, the “control region” limitation is satisfied, irrespective of whether a single operon was used to construct it.<sup>5</sup>

In the context of the patented invention, the term “control region” describes functional control elements involved in the production of a protein and is directed to a sequence of DNA, not a method for constructing such a sequence. Rather than requiring a limitation on the method of constructing a control region, the patent appears to preclude one. In the section titled “The Control Elements,” the specification discloses using a control region comprising “control elements” derived from a bacteriophage infective for *E. Coli*. '362 Patent, col. 8, ll. 45-48. The specification further suggests obtaining control elements from “other operons or portions thereof.” *Id.* at col. 8, ll. 56-60.

Likewise, the prosecution history shows that a control region may be constructed portion-by-portion. In an amendment submitted to the PTO on January 11, 1996, the patentee explained: “The specification quite clearly supports the preparation of expression control regions wherein the elements are taken from various sources, including partial or total synthesis, and operatively linked by generally well-known ligation means to provide *functional*, homologous expression control region as such.” Amendment submitted to U.S. Patent and Trademark Office, U.S. Application Serial No. 08/434,321, at 6 (Jan. 11, 1996).

Thus, all the control elements of a single operon need not be derived from a single operon to comprise a control region in the context of the patented invention, and the district court's contrary conclusion was in error. For purposes of satisfying the control region limitation, some control elements may be physically derived from an operon and other control elements may be chemically synthesized or physically derived from a different operon, as long as the sequence of the three control elements in the control region correspond to the sequence found in a single operon. This court thus finds error in the district court's requiring that each control element be unique, so as to make it possible to determine whether it was derived from the same operon as the other control elements. Simply put, the method by which the control region is constructed and the sources from which it is derived are inapposite, thus negating any requirement that such methods or sources be discernable from the sequence of the control elements. Based on the foregoing definitions of the terms "ribosome binding site" and "control region," this court holds that the asserted claims require at least a promoter, operator, and a ribosome binding site that, when transcribed

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into mRNA, is bound by the ribosome and is necessary and sufficient for the initiation of translation. The promoter, operator, and ribosome binding site must correspond to the promoter, operator, and ribosome binding site found in a single operon; the sources and methods used to construct the control region are irrelevant. There is no dispute that Amgen's accused plasmid contains promoter and operator sequences that correspond to the sequences found in the lambda operon. The key infringement issue thus becomes whether, in the Amgen cloning vehicle and process, the sequences in the ribosome binding site area that correspond to the sequences in the lambda operon—the AGGAG Shine-Dalgarno sequence and the ATG start codon—comprise the element of the control region that binds the ribosome and is (along with the operator and promoter) necessary and sufficient for the initiation of translation. On the basis of expert testimony, Genentech argues that these sequences are sufficient to bind the ribosome and initiate translation. For its part, Amgen asserts that these eight base pairs are not sufficient to bind the ribosome and, along with the operator and promoter, initiate translation. This dispute precludes granting summary judgment of non-infringement.<sup>6</sup>

Furthermore, summary judgment procedures required the district court to draw reasonable inferences in favor of Genentech, the non-movant. *Viskase Corp. v. Am. Nat'l Can Co.*, 261 F.3d 1316, 1324, 59 USPQ2d 1823, 1828 (Fed. Cir. 2001). Accordingly, in light of the factual dispute and the erroneous claim construction, this court vacates the summary judgment and remands for a determination of infringement under the revised claim construction set forth above. *Lampi Corp. v. Am. Power Prods., Inc.*, 228 F.3d 1365, 1376, 56 USPQ2d 1445, 1454 (Fed. Cir. 2000).

#### IV.

In its Fed. R. Civ. P. 56(f) motion, Genentech sought access to the sequence of the entire control element in Amgen's plasmid, as well as the process by which Amgen constructed their control region. Genentech contended that it needed to compare the entire control region of Amgen's plasmid to control elements of other known bacterial genetic material, such as lambda, to determine whether Amgen's control region corresponded to the control element of a single operon. Genentech requested laboratory notebooks, internal company

memoranda, meeting minutes, test data, and notes relating to the specific components of Amgen's plasmid.

[2] A sequence comparison of the relevant RBSs, which already are available to Genentech, is sufficient to determine whether the control region of Amgen's plasmid falls within the scope of Genentech's claimed "control region." For example, a sequence comparison of the RBSs is sufficient for one to be able to see whether Amgen's control region is "homologous" to lambda's control region. The P and O regions of Amgen's plasmid clearly are derived from lambda (the sequences are identical), but Amgen's RBS linker sequence has base pairs that diverge in number and identity from (i.e., are not homologous with) lambda's RBS. Additional discovery on this issue is not necessary to determine infringement. Consequently, the district court did not abuse its discretion when it denied Genentech's motion for discovery regarding the entire sequence of, or method of making, the control element within Amgen's plasmid.

#### V.

The district court precluded Genentech from proceeding on a theory of infringement under the doctrine of equivalents because Genentech did not expressly include that theory in a claim chart, as strictly required under Civil Local Rule 16-9 (Rule 16-9) at the time. *Original Summary Judgment Order* at 7. As noted by the district court, under Rule 16-9(c), the patentee may amend its claim chart: (1) on stipulation of the parties; (2) upon a showing of excusable subsequent discovery of new information;

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or (3) upon a showing of clearly excusable neglect. *Id.*

[3] Genentech claims that it understood Local Rule 16-9 to require that the patent holder prepare a claim chart listing whether the claimed infringement is "literal *or* under the doctrine of equivalents." In other words, Genentech asserts that it reasonably thought that it was required to choose between the two types of infringement when it filed its claim chart in October 1997. Based on this, Genentech argues that it believed in good faith that the claim chart did not constitute its final commitment to the theories of infringement that it could pursue. Genentech notes that it prepared the claim chart well before the court's claim construction in May 1999. Genentech's argument flies in the face of common sense and years of Federal Circuit precedent. Genentech must have known that it could initially assert both types of infringement. Furthermore, if it had any questions regarding the claim chart, it could have asked the court for a clarification.

Genentech does not assert any satisfactory reasons as to why it should be allowed to amend its claim chart. As noted by the district court, Genentech first explicitly alluded to the doctrine of equivalents more than a year after it filed its complaint and after the court issued its claim construction order. Rule 16-9 requires that the patentee give notice to the accused infringer of its infringement theories before the claim construction hearing. In this regard, Genentech contends that Amgen had notice of its doctrine of equivalents theory since 1997. For example, the parties made reference to "prosecution history estoppel" in a joint management statement in 1997. Moreover, Amgen acknowledged the relevance of doctrine of equivalents in its opposition to Genentech's motion to compel documents in 1998, and continued to assert arguments regarding the doctrine of

equivalents as late as January 2000. Finally, according to Genentech, Amgen suffered no prejudice from Genentech's failure to amend its claim chart.

Genentech's argument is unavailing because “[u]nlike the liberal policy for amending pleadings, the philosophy behind amending claim charts [under Rule 16-9] is decidedly conservative and designed to prevent the ‘shifting sands’ approach to claim construction.” *Atmel Corp. v. Info. Storage Devices, Inc.*, No. C 95-1987, 1998 WL 775115, at \*2 (N.D. Cal. Nov. 5, 1998). Furthermore, this court defers to the district court when interpreting and enforcing local rules so as not to frustrate local attempts to manage patent cases according to prescribed guidelines. In reviewing a district court's exercise of discretion, this court determines “whether (1) the decision was clearly unreasonable, arbitrary, or fanciful; (2) the decision was based on an erroneous conclusion of law; (3) the court's findings were clearly erroneous; or (4) the record contains no evidence upon which the court rationally could have based its decision.” *In re Cambridge Biotech Corp.*, 186 F.3d 1356, 1369, 51 USPQ2d 1321, 1329 (Fed. Cir. 1999). In this case, Genentech does not point to any specific legal or factual error in the district court's decision. While the record shows ample reasons for the district court to permit Genentech to amend its claim chart, our standard of review on this issue does not require reversal in the presence of reasons to permit amendments. Even a determination that the district court's ruling was erroneous does not require reversal. Only if the ruling is found to be clearly erroneous is reversal mandated. The district court's determination on this issue was not clearly erroneous. Accordingly, the district court did not abuse its discretion by enforcing the Local Rule and precluding Genentech from asserting infringement under the doctrine of equivalents when Genentech did not include that theory in its claim chart.

### CONCLUSION

This court vacates the summary judgment that Amgen does not infringe the '362, '619 and '013 patents. This court remands the case to the district court for further proceedings to determine whether Amgen infringes the '362, '619 and '013 patents. Further, this court affirms the trial court's ruling on Genentech's noncompliance with the Local Rule.

### COSTS

Each party shall bear its own costs.

***AFFIRMED-IN-PART, VACATED-IN-PART, and REMANDED.***

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### Footnotes

1 Under this construction, “hybrid” control regions are outside the scope of the claims. *Id.* at 21. On appeal, neither party challenges this claim construction. A hybrid control region is one comprised of control elements from various operons, in which the sequence of the control elements does not correspond to the sequence found in a single operon.

2 In ruling on the summary judgment motion, the district court (Judge Alsup) did not address whether a “homologous control region” requires an intact control region endogenous to the host. *Genentech, Inc. v. Amgen, Inc.*, No. C 96-03752 WHA, slip op. at 10 (N.D. Cal. Aug. 28, 2000) (*Original Summary Judgment Order*); *Amended*

*Summary Judgment Order at 12.*

3 Amgen responds that the district court did not limit the claims to a particular method of constructing an operon, but instead properly construed the “control region” limitation to require that the control elements be endogenous to the host cell. This court cannot accept Amgen's interpretation of the Summary Judgment Order, as Judge Alsup's rejection of Genentech's proposed construction of the term “ribosome binding site” on the basis of inability to determine from which operon the ribosome binding site was derived necessarily limits the methods by which a control region is constructed.

4 It appears that the confusion between the sequence of the control region and the method for constructing it arose from the original claim construction's requirement that the control region “be taken from a single operon.” This construction is only correct to the extent that it is understood to refer to a control region containing a sequence that corresponds to the sequence of the control elements in a single operon, so that it is not a hybrid control region.

5 The district court (Judge Smith) rejected Amgen's contention that the control region must be intact, i.e., identical to a control region native to the untransformed host. Judge Smith found that the specification and file history in fact refuted Amgen's proposed limitation requiring the use of a single operon “taken intact” to construct the control region. Rather, she noted: “What these examples disclosed in the patent suggest is that some variation from the native control region of the untransformed host is permissible as long as the control region remains operable. In short, the control region need not be ‘intact.’” *Id.* at 19-20.

6 As an alternative ground for affirmance, Amgen argues that even if its accused plasmid contains a control region, it does not meet the “homologous control region” limitations of the asserted claims in the '362 and '619 patents. Aside from recasting its arguments concerning the “ribosome binding site” and “control region” limitations in a different context, Amgen essentially argues that because its plasmid differs from the lambda operon in areas other than the control elements (P, O, and RBS), its control region is not endogenous to lambda and thus not a homologous control region. This court leaves the consideration of this non-infringement argument to the district court to consider in the first instance.

**- End of Case -**

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## Enzymes for Bioanalytical Chemistry

### Glycosidases

#### Introduction

Glycosylation of biomolecules, especially proteins, is of major importance for their biology. Thus investigation of glycosylation patterns is crucial in life sciences. As bacteria lack the ability to glycosylate proteins, glycosylation is a major issue for use of recombinant proteins. See hydrolysis by glycosidases is applied for characterization of glycoproteins.

Fluka offers a wide range of Glycosidases. Please take a look at the table below:

**Table. Glycosidases** Note

Catalog No.	Product Name
<b><u>10065</u></b>	alpha-Amylase from <i>Aspergillus oryzae</i> , powder ~30 U/mg
<b><u>10067</u></b>	alpha-Amylase from <i>Bacillus licheniformis</i> , ~2 U/mg
<b><u>10068</u></b>	alpha-Amylase from <i>Bacillus</i> sp., ~1000 U/mg
<b><u>10069</u></b>	alpha-Amylase from <i>Bacillus subtilis</i> , ~380 U/mg
<b><u>10070</u></b>	alpha-Amylase from <i>Bacillus subtilis</i> , powder, ~50 U/mg
<b><u>10094</u></b>	alpha-Amylase from hog pancreas, ~20 U/mg
<b><u>10080</u></b>	alpha-Amylase from hog pancreas, powder ~50 U/mg
<b><u>10092</u></b>	alpha-Amylase from human saliva, ~100 U/mg
<b><u>10095</u></b>	alpha-Amylase, DFP treated from hog pancreas, ~700 U/mg
<b><u>10090</u></b>	alpha-Amylase, PMSF treated from hog pancreas, ~20 U/mg
<b><u>63412</u></b>	alpha-Glucosidase from yeast, 65 U/mg
<b><u>10114</u></b>	Amyloglucosidase from <i>Aspergillus niger</i> , >50 U/mg, ~20 U/mg
<b><u>10113</u></b>	Amyloglucosidase from <i>Aspergillus niger</i> , ~120 U/mg
<b><u>10115</u></b>	Amyloglucosidase from <i>Aspergillus niger</i> , lyophilized, ~120 U/mg

**EXHIBIT 2**

<b><u>49103</u></b>	beta-1,3-D-Glucanase from <i>Helix pomatia</i> , ~0.3 U/mg
<b><u>10100</u></b>	beta-Amylase from barley, ~15 U/mg
<b><u>10112</u></b>	beta-Amylase from sweet potato, package with 15 mg
<b><u>48275</u></b>	beta-Galactosidase from <i>E. coli</i> , lyophilized, stab., ~14
<b><u>48274</u></b>	beta-Galactosidase from <i>E. coli</i> , ~450 U/mg
<b><u>48277</u></b>	beta-Galactosidase from <i>Kluyveromyces fragilis</i> , ~5 U/
<b><u>49101</u></b>	beta-Glucanase from <i>Aspergillus niger</i> , ~1 U/mg
<b><u>49106</u></b>	beta-Glucanase from <i>Bacillus subtilis</i> , ~1 U/mg
<b><u>49290</u></b>	beta-Glucosidase from almonds lyophilized powder, ~6
<b><u>49315</u></b>	beta-Glucuronidase <i>E. coli</i> K12, stab., 100 U/ml, vial 1
<b><u>49312</u></b>	beta-Glucuronidase from <i>E. coli</i> , ~1.5 U/mg
<b><u>49313</u></b>	beta-Glucuronidase from <i>E. coli</i> , lyophilized, ~4 U/mg
<b><u>22180</u></b>	Cellulase from <i>Aspergillus niger</i> , ~0.1 U/mg
<b><u>22178</u></b>	Cellulase from <i>Aspergillus niger</i> , ~0.5 U/mg
<b><u>22175</u></b>	Cellulase from <i>Humicola insolens</i> , ~0.02 U/mg
<b><u>22173</u></b>	Cellulase from <i>Trichoderma reesei</i> , ~1 U/mg
<b><u>22725</u></b>	Chitinase from <i>Streptomyces griseus</i> , ~70 U/g
<b><u>27038</u></b>	Chondroitinase ABC from <i>Proteus vulgaris</i> , lyophilized,
<b><u>27540</u></b>	Clara-Diastase, mixture of enzymes, powder
<b><u>31402</u></b>	Dextranase from <i>Paecilomyces lilacinus</i> , ~60 U/mg
<b><u>33470</u></b>	Diastase from malt, >2300 U/gamylase activity
<b><u>48235</u></b>	Galactomannanase from <i>Aspergillus niger</i> , ~0.2 U/mg
<b><u>49291</u></b>	Glucosidase from <i>Aspergillus niger</i> , ~60 U/g
<b><u>53718</u></b>	Hyaluronidase from bovine testes, ~0.25 U/mg

<b><u>53708</u></b>	Hyaluronidase from ovine testes, lyophilized, ~0.25 U/r
<b><u>53725</u></b>	Hyaluronidase from Streptomyces hyalurolyticus, ~0.1
<b><u>57620</u></b>	Inulinase from Aspergillus niger, ~17 U/mg
<b><u>57628</u></b>	Invertase from baker's yeast ( <i>S. cere-visiae</i> ), ~300 U/r
<b><u>57629</u></b>	Invertase from baker's yeast, powder, ~100 U/mg
<b><u>72201</u></b>	Neuraminidase from Clostridium perfringens, ~1 U/mg
<b><u>72202</u></b>	Neuraminidase from Clostridium perfringens, ~3 U/mg
<b><u>72197</u></b>	Neuraminidase from Vibrio cholerae, ~2 U/ml
<b><u>76286</u></b>	Pectin Esterase from orange peel, ~55 U/mg
<b><u>76287</u></b>	Pectinase from Rhizopus sp., ~20 U/mg
<b><u>76285</u></b>	Pectinase from Rhizopus sp., ~5 U/g
<b><u>82577</u></b>	Pullulanase from Bacillus sp., powder, ~1 U/mg
<b><u>86250</u></b>	Taka-Diastase from Aspergillus oryzae, powder, ~1.5 U
<b><u>86247</u></b>	Taka-Diastase from Aspergillus oryzae, powder, ~40 U
<b><u>95592</u></b>	Xylanase from bacteria, ~3 U/mg
<b><u>95595</u></b>	Xylanase from Trichoderma viride, 2.5 U/mg

Note: In general our activity U is based on the amount of enzyme which reduces 1  $\mu$ mol dihydroxyacetonephosphate per minute at 25 °C. For a precise definition per enzyme, look at the product information sheet.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) Ding et al.

Serial No.: 09/917,376

Filed: July 28, 2001

For: Thermal Tolerant Avicelase  
From *Acidothermus*  
*cellulolyticus*

Group No.: 1652

Examiner: Swope,  
Sheridan

Confirmation No. 9956

CERTIFICATE OF MAILING

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited in the United States Postal Service on the date shown below with sufficient postage as U.S. Express Mail Label No. E1820326198US, in an envelope addressed Assistant Commissioner for Patents, Washington D.C. 20231.

Date

Heather Bailey

BOX: FEE AMENDMENT  
Commissioner For Patents  
Washington, D.C. 20231

RULE 132 DECLARATION

This Declaration is presented to provide the Examiner with additional evidence that deserves consideration in traversing one or more of the rejections presented in an Office Action dated August 1, 2002.

1. The undersigned individuals are Michael Edward Himmel, PhD, and William S. Adney, who are also named inventors in the above-identified patent application.
2. Both of the undersigned individuals are employed as scientists at the National Renewable Energy Laboratory located in Golden, Colorado. Dr. Himmel holds

the position of Senior Scientist II, and Mr. Adney that of Senior Scientist. Both men are engaged in research that includes the identification, isolation and cloning of cellulases.

3. Exhibit A to this Declaration is a *curriculum vitae* for Dr. Himmel. Exhibit A shows that Dr. Himmel has worked for more than twenty-two years in the field of microbiology and biochemistry. Dr. Himmel has received prestigious awards, such as the American Chemical society Outstanding Service Award for 1990, and he has authored at least one hundred seventy three publications. Dr. Himmel is frequently asked to chair and/or organize portions of scientific meetings.

4. Exhibit B to this Declaration curriculum vitae for Mr. William S. Adney. Exhibit B shows that Mr. Adney has a Masters of Science in Microbiology and has worked in the field of microbiology since 1979. Mr. Adney has authored over 49 journal and symposium papers, in addition to seventy-five meetings abstracts and proceedings.

5. The undersigned have reviewed the claims, as amended, in addition to the office action dated August 1, 2002.

6. Dr. Himmel is named as an inventor in US 5,110,735 (Tucker 1992), and US 5,366,884 (Adney 1994). Mr. Adney is named as an inventor in Adney 1994.

7. Pages 9-10 of the Office Action, and likewise pages 10-14, contain statements to the effect that Tucker et al. 1992 and Adney et al. 1994 disclose a high molecular weight endoglucanase ranging from 156 to 203 kDa, and the deduced molecular weight of the AvIII cellulase is 105 kDa. Thus, the office action alleges that the AvIII cellulase is the same high molecular weight cellulase taught in Tucker et al. 1992 and Adney et al. 1994, because glycosylation accounts for the weight difference.

8. We emphatically disagree with the allegations observed in Paragraph 7 because *Acidothermus* and most other bacteria that secrete proteins do not glycosylate them. Glycosylation of secreted extracellular enzymes is common in eukaryotes, but bacteria use other mechanisms to transport proteins outside the cell wall. Bacterial enzymes, such as cellulases, are not glycosylated. Therefore, the 105 kDa Avicellase reported in this specification cannot be the endoglucanase(s) taught by Tucker 1992 or Adney 1994 on the basis of molecular weight alone.

9. The three enzymes taught by Tucker 1992 and Adney 1994 were all identified as endoglucanases, which are a specific class of cellulase. Our continuing research has shown that the cellulase derived from AvIII has an activity profile consistent with exoglucanase activity, and not endoglucanase activity. Avicellases are exoglucanases and do not have activity on the same substrate that endoglucanases do. Endoglucanases hydrolyze carboxymethylcellulose (CMC) and exoglucanases do not. Thus, the AvIII-derived exoglucanase cannot be the endoglucanase identified in Tucker 1992 and Adney 1994.

10. Example 1 beginning on page 31 of the present specification describes the way in which we created a phage library from genomic DNA of *Acidothermus*, transfected *E. coli*, and initially screened the clones using CMC-Congo Red plates. Positive clones for endoglucanase activity showed up as unstained plaques on a red background. Sequencing data from primer-walking and subclones identified an open reading frame identified as AvIII, and a C-terminal fragment of another gene. It was this other gene that had endocellulase activity, not AvIII. Thus, in screening for

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endoglucanases from our library, we serendipitously identified an exoglucanase. The endoglucanase activity reported in Example 1 does not show endoglucanase activity for the AviiII-derived cellulase.

11. For the reasons stated above, and in addition being members of the research teams involved with Tucker 1992 and Adney 1994, we are convinced that the high molecular weight cellulase described in Tucker 1992 and Adney 1994 cannot be the AviiII-derived cellulase taught in the present specification.

12. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 12-24-02By: Michael E. Himmel

Michael E. Himmel

Date: 12-24-02By: William S. Adney

William S. Adney

## CURRICULUM VITAE - Brief

### MICHAEL EDWARD HIMMEL

#### OFFICE ADDRESS:

Biotechnology Center for Fuels and Chemicals  
National Renewable Energy Laboratory (NREL)  
1617 Cole Blvd.  
Golden, Colorado 80401

(303) 275-3887 voice; (303) 275-3799 FAX; email: himmelm@tcplink.nrel.gov

#### HOME ADDRESS:

9202 W. Hialeah Place  
Littleton, CO 80123  
(303) 972-4804

#### PRESENT POSITION:

Senior Scientist II

- o Enzyme Technology Team Leader - supervise 6 to 8 staff scientists and postdocs doing biochemistry and cloning (19 yrs experience).
- o Adjunct Faculty: Department of Agricultural and Chemical Engineering at CSU, Fort Collins, CO.

#### EDUCATION:

- o PhD, Biochemistry, Colorado State University, Fort Collins, CO, December 1980
- o BS, Chemistry major and Biology minor, Magna Cum Laude, University of Northern Colorado, Greeley, Colorado, June 1974.

#### AWARDS:

- NREL/MRI 1993 Staff Award for Outstanding Personal Performance
- NREL/MRI 1997 Staff Award for Outstanding Team Performance

#### PROFESSIONAL ACTIVITIES:

- o Member, American Chemistry Society
- o Member, American Society of Microbiology
- o Member, Sigma Xi, Scientific Research Society
- o Member, Planning Committee, Symposium for Biotechnology of Fuels & Chemicals
- o Ad hoc Reviewer: *Appl. Biochem. Biotechnol.*, *J. Chromatogr.*, *Bioresource Technol.*, ACS Books, USDA SBIR, Consortium for Plant Biotechnology Research, DOE OS and DOE BES

#### PUBLICATIONS AND PATENTS:

Full length scientific papers: 132

Scientific posters and presentations: 160

Patents, PCTs, and DOE Invention Reports: 20

#### HOBBIES:

High plains archeology, photography, antique firearms, antique watches.

#### PERSONAL DATA:

Health: Variable

Marital Status: Married

Age: 50

# MICHAEL E. HIMMEL

## OBJECTIVES

---

Engineering improved industrial biocatalysts for biomass conversion using a knowledge-based approach to enzyme and cell function.

## WORK EXPERIENCE

---

1998-present    National Renewable Energy Laboratory    Golden, CO  
*Principal Scientist*

- Focus Team Leader – Enzyme Technology team (10 professional staff and 6 university and industry subcontractors).

1985 - 1999    National Renewable Energy Laboratory    Golden, CO  
*Senior Scientist II*

Team Leader – Enzyme Technology team (manage 7 scientists doing the biochemistry and cloning/expression of selected glycosyl hydrolases).

1980 - 1985    Solar Energy Research Institute    Golden, CO  
*Senior Scientist I – Staff Microbiologist*

Biochemist working on the isolation and characterization of new microorganisms and enzymes from extreme natural environments.

## EDUCATION

---

1974 - 1980    Colorado State University    Fort Collins, CO  
*PhD/Biochemistry*

1970 - 1974    University of Northern Colorado    Greeley, CO  
*BS/Chemistry with Biology Minor (Magna Cum Laude)*

## PATENTS AND PUBLICATIONS

---

Full length scientific papers, posters, presentations, patents ~300

## AWARDS RECEIVED

---

1990 – American Chemical Society Outstanding Service Award

1992 – NREL/MRI Staff Award for Outstanding Performance

1998 – NREL/MRI Staff Award for Outstanding Team Performance

2002 – NREL/MRI H. Hubbard Award for Research Management

## CIRICULUM VITAE - BRIEF

**Michael E. Himmel, PhD**

### EDUCATION

PhD 1980 Biochemistry, Colorado State University, Fort Collins, CO.

BS 1974 Chemistry Major, University of Northern Colorado, Greeley, CO.

### QUALIFICATIONS AND EXPERIENCE

Dr. Himmel has 26 years of progressive experience in conducting, supervising, and planning research in protein biochemistry, recombinant technology, enzyme engineering, new microorganism discovery, and the physicochemistry of macromolecules. Although known internationally for his work in cellulase biochemistry and lignin characterization, he has directly supervised projects that target the isolation of new, thermotolerant microorganisms; as well as the cloning and application of the thermal stable enzymes they produce. He has also supervised research that targets the application of site-directed-mutagenesis and rational protein design to the stabilization of important industrial enzymes. As might be expected, the development of new assays for hydrolytic enzymes is an important aspect of such enzyme engineering work, and he has contributed many new methods to the literature. Dr. Himmel also initiated and managed the first Chemical Analysis and Testing team in the DOE OTT Biofuels program at NREL. This team conducted advanced chemical analyses and quality control operations for a Division of 100 staff. Dr. Himmel's work history also encompasses pilot plant scale experience, as he designed various facilities and novel equipment for process biotechnology research, including a series of novel high solids fermenters for methane production, a paddle type reaction/fermentation system for high solids processes, and several pilot plant scale, preparative chromatography systems. More recently, Dr. Himmel was co-investigator on a successful proposal funded by DOE OS Nanotechnology that will explore the application of novel protein scaffolds to create self-assembling arrays of quantum dots.

Dr. Himmel currently manages the DOE OTT Biofuels Enzyme Technology team of 10 staff scientists and directly monitors 5 DOE/NREL subcontracts at universities and biotechnology companies. In 1998, the Enzyme Technology team received the NREL Research Team Award. The daily interaction with junior and senior engineers, program managers and planners, and NREL support staff has always been an important element of his job description. Apart from this, Dr. Himmel serves as adjunct faculty to the Agricultural and Chemical Engineering Department at Colorado State University. In this role, he has served on five doctoral committees. Dr. Himmel has organized 4 international American Chemical Society symposia, each resulting in ACS books (i.e., Vols. 460, 516, 586, 769) edited by Dr. Himmel. During the last decade, Dr. Himmel has contributed over 290 journal papers and meeting abstracts, as well as 16 NREL/DOE patents and patent applications. Dr. Himmel is currently Chairman of the new Gordon Research Conference on "Cellulases and Cellulosomes."





**MICHAEL EDWARD HIMMEL  
PUBLICATION LIST  
JOURNAL AND SYMPOSIUM PAPERS**

1. "Arginine Deiminase from *Mycoplasma arthritidis*: Properties of the Enzyme from Log Phase Cultures", J.L. Weickmann, M.E. Himmel, P.G. Squire and D.E. Fahrney, J. Biol. Chem., **253**, 6010-6015 (1978).
2. "Isolation and Physical Characterization of Bovine Lens Crystallins", S-H. Chiou, P. Azari, M.E. Himmel and P.G. Squire, Int. J. Pept. Protein Res., **13**, 409-417 (1978).
3. "Arginine Deiminase: Demonstration of Two Active Sites and Possible Half-of-the-Sites Reactivity", J.L. Weickmann, M.E. Himmel, D.W. Smith and D.E. Fahrney, Biochem. Biophys. Res. Commun., **83**, 107-113 (1978).
4. "Evidence for the Alteration of the Membrane-bound Ribosomes in *Micrococcus luteus* Cells Exposed to Lead", W. Barrow, M.E. Himmel, P.G. Squire and T.G. Tornabene, Chemical-Biological Interactions, **23**, 387-397 (1978).
5. "Hydrodynamics and Protein Hydration", P.G. Squire and M.E. Himmel, Arch. Biochem. Biophys., **196**, 165-177 (1979).
6. "Physical-Chemical Studies of Bacterial Macromolecules and Model Systems", M.E. Himmel, Ph.D. Thesis, Colorado State University, Fort Collins, CO, 1980.
7. "High Pressure Gel Permeation Chromatography of Native Proteins on TSK-SW Columns", M.E. Himmel and P.G. Squire, Int. J. Pept. Protein Res., **17**, 365-373 (1981).
8. "High Pressure Size Exclusion Chromatography of Sea Worm Chlorocruorin and Other Large Proteins, Viruses and Polysaccharides on a TSK G5000 PW Preparative Column", M.E. Himmel and P.G. Squire, J. Chromatogr., **210**, 443-452 (1981).
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14. "A Novel Reaction Bomb Sampling Device for Microparticulate Slurries", M.E. Himmel, K.K. Oh, M. Tucker, and J. Janssens, Biotechnol. Bioeng., **25**, 619-622 (1983).
15. "High Performance Size Exclusion Chromatography of Low-Molecular Weight Lignins and Model Compounds", M.E. Himmel, K.K. Oh, D.W. Sopher and H.L. Chum, J. Chromatogr., **267**(2), 249-265 (1983).
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30. "Some Aspects of Lignin Characterization of HPSEC Using Styrene-Divinylbenzene Copolymer Gels", H.L. Chum, D.K. Johnson, M.P. Tucker and M.E. Himmel, Holzforschung, **41**, 97-108 (1987).
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45. "Alkaline SEC of Lignins and Coal Extracts using Cross-Linked Dextran Gels", M.E. Himmel, K.K. Oh, D.R. Quigley, and K. Grohmann, J. Chromatogr., **467**(1), 309-314 (1989).
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50. "Characterization of Polysaccharidase Activity Optima in the Anaerobic Digestion of Municipal Solid Waste", W.S. Adney, C.J. Rivard, K. Grohmann and M.E. Himmel, Biotech. Lett., **3**, 207-210 (1989).
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52. "Biodegradation of Plastics", C.J. Rivard, M.E. Himmel, and K. Grohmann, in "Assessment of Biobased Materials", H.L. Chum (ed.), Chapter 8, SERI/TR-234-3610, 1989; pp 8-1/8-6.
53. "Dilute Acid Pretreatment of Short Rotation Woody and Herbaceous Crops", R. Torget, P. Werdene, M. Himmel, and K. Grohmann, Appl. Biochem. Biotechnol., **24/25**, 115-126 (1990).

54. "Monoclonal Antibody Affinity Purification of *Trichoderma reesei* EG I", R.A. Nieves, M.E. Himmel and R.P. Ellis, Appl. Biochem. Biotechnol., **24/25**, 397-406 (1990).
55. "Cellulase Production by *Acidothermus cellulolyticus*", J. Linden, M. Shiang, A. Mohagheghi, C. Rivard, K. Grohmann, and M.E. Himmel, Appl. Biochem. Biotechnol., **24/25**, 223-235 (1990).
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### ***BOOKS EDITED***

1. *"Enzymes in Biomass Conversion,"* (G.F. Leatham and M.E. Himmel, eds.), ACS Series **460**, American Chemical Society: Washington, DC, 1991.
2. *"Biocatalyst Design for Stability and Specificity,"* (M.E. Himmel and G. Georgiou, eds.), ACS Series **516**, American Chemical Society: Washington, DC, 1993.
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4. "Glycosyl Hydrolases for Biomass Conversion," (M.E. Himmel, J.O. Baker, and J. Saddler, eds.), ACS Series **769**, American Chemical Society: Washington, DC, 2000.

### ***VIDEO/ANIMATION***

1. "Cellulase Animation," M. Himmel, S. Decker, W. Adney, J. Baker, Run time 11 min., NREL, Golden, CO. 2000. Copyright DOE/MRI PAU2-568-354.
2. "Cellulase Animation - Brief," Michael Himmel and Dan Seely, Run time 6 min., NREL, Golden, CO. 2000. Copyright DOE/MRI.
3. "Simultaneous Saccharification Fermentation Animation," Michael Himmel and Dan Seely, Run time 15 min., NREL, Golden, CO. In preparation

## **MEETING ABSTRACTS AND PROCEEDINGS**

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88. "SSF Comparison of Selected Woods from Southern Saw Mills", L.R. Ponfick, T. Vinzant, N.J. Nagle, T.I. Ehrman, K.M. Magill, J.B. Reynolds, and M.E. Himmel, Poster No. III. 19, The Fifth Annual Colorado Biotechnology Symposium organized by CIRB, Colorado State University, Fort Collins, CO, September (1992).
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90. "Cellulase Research for DOE", M.E. Himmel, The 1992 Automotive Technology Development Contractors Coordination Meeting, Dearborn, MI, November 2-5, 1992.
91. "Monoclonal Antibodies Against Elk, Mule Deer, White Tailed Deer, and Antelope Albumins", R.P. Ellis, R.J. Todd, K.L. Cannizzo, W.J. Adrian, E.L. Belden, R.A. Schamber, R.A. Nieves, and M.E. Himmel, The Northwest Association of Forensic Scientists, Portland, OR, October, 1992.
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93. "Endoglucanase E1 from *Acidothermus cellulolyticus* is a Family A Cellulase," S.R. Thomas, R.A. Laymon, M.P. Tucker, T.B. Vinzant, and M.E. Himmel, "Recombinant DNA II", 1993 Engineering Foundation Meeting, Palm Coast, FL, February, 1993.
94. "Visualization of Cellulases Bound to Cellulose Protofibrils: Evidence for Endo/Exo Synergism," Rafael A. Nieves, Robert P. Ellis, and Michael E. Himmel, The symposium on "Bioconversion for Fuels", The 1993 Annual American Chemical Society meeting, Denver, CO, March, 1993.
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100. "SSF Comparison of Selected Woods from Southern Saw Mills", T.B. Vinzant, L.R. Ponfick, N.J. Nagle, C.I. Ehrman, K.M. Magill, J.B. Reynolds, and M.E. Himmel, The Fifteenth Symposium on Biotechnology for Fuels and Chemicals, Colorado Springs, CO, May, 1993.
101. "Purification and Characterization of Acetyl-Xylan Esterase from *Aspergillus niger*," James C. Linden, Meropi Samara, Miklos Pecs, Ellen Thomas, Michelle Joy, William Adney, and Michael E. Himmel, The Fifteenth Symposium on Biotechnology for Fuels and Chemicals, Colorado Springs, CO, May, 1993.
102. "Evaluation of Discrete Cellulase Enzyme Activities from Anaerobic Digester Sludge Fed a Municipal Solid Waste Feedstock," C.J. Rivard, R.A. Nieves, N.J. Nagle, and M.E. Himmel, The Fifteenth Symposium on Biotechnology for Fuels and Chemicals, Colorado Springs, CO, May, 1993.
103. "Cellulase Research for the DOE Biofuels Program," M.E. Himmel, N.D. Hinman, and S.R. Thomas, distributed at The IEA-Symposium on Biotechnology for the Conversion of Lignocellulosics, Helsinki, FINLAND, June 6-9, 1993.
104. "The Cellulase Synergistic Effect: Assembly of Optimal Biochemical Systems", S.R. Thomas, J.O. Baker, W.S. Adney, R.A. Nieves, M.P. Tucker, T.B. Vinzant, D.B. Wilson, D.E. Eveleigh, and M.E. Himmel, The Society for Industrial Microbiology & Canadian Society of Microbiologists, Toronto, CANADA, July, 1993.
105. "Initial Approaches to Artificial Cellulase Systems for Conversion of Biomass to Ethanol," S.R. Thomas, R.A. Laymon, Y.-C. Chou, M.P. Tucker, T.B. Vinzant, W.S. Adney, J.O. Baker, R.A. Nieves, and M.E. Himmel, The symposium on *Advances in the Bioconversion of Lignocellulosics*, Presented as an oral paper at The 1994 Annual American Chemical Society Meeting, San Diego, CA, March, 1994.
106. "The Cellulase Synergistic Effect: Binary and Ternary Systems," J.O. Baker, S.R. Thomas, W.S. Adney, R.A. Nieves, and M.E. Himmel, The symposium on *Enzymic Degradation of Insoluble Polysaccharides*, Presented as an oral paper at The 1994 Annual American Chemical Society Meeting, San Diego, CA, March, 1994.
107. "Immunogold Labeling of a Recombinant Thermophilic Bacterial Endoglucanase Expressed in *Escherichia coli* Using Monoclonal Antibody," R.A. Nieves, M.P. Tucker, R.A. Laymon, S.R. Thomas, and M.E. Himmel, Presented as an oral paper at the 1994 Annual Meeting of the American Society of Microbiology, Las Vegas, May, 1994.
108. "Quantitation of *Acidothermus cellulolyticus* E1 endoglucanase and *Thermomonospora fusca* E3 Exoglucanase Using Enzyme-Linked Immunosorbent Assays (ELISA)," R.A. Nieves, Y.-C. Chou, D.B. Wilson, S.R. Thomas, and M.E. Himmel, Presented as a poster at The Sixteenth Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN, May, 1994.
109. "Horsepower Requirements for High Solids Anaerobic Digestion", C.J. Rivard, B.X. Kay, D.X. Kerbaugh, N.J. Nagle, and M.E. Himmel, Presented as a poster at The Sixteenth Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN, May, 1994.
110. "*Acidothermus cellulolyticus* E1 Endoglucanase: Characteristic of Native and Recombinant Forms", S.R. Thomas, W.S. Adney, J.O. Baker, Y.-C. Chou, R. Laymon, R.A. Nieves, M.P. Tucker, T.B. Vinzant, and M.E. Himmel, Presented as a poster at The Sixteenth Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN, May, 1994.
111. "The Gene Encoding the *Acidothermus cellulolyticus* E1 Endoglucanase", R.A. Laymon, S.R. Thomas, T.B. Vinzant, W.S. Adney, and M.E. Himmel, Poster No. 21, The 7th Annual Colorado Biotechnology Symposium organized by CIRB, Colorado State University, Fort Collins, CO, September, 1994.
112. "Kinetic Comparison of  $\beta$ -D-glucosidases of Industrial Importance", W.S. Adney, J.O. Baker, T.B. Vinzant, S.R. Thomas, and M.E. Himmel, Presented as an oral paper at the 1995 Annual Meeting of the American Chemical Society, Anaheim, CA, April, 1995.
113. "Cloning and Expression of the *Acidothermus cellulolyticus* E1  $\beta$ -1,4-Endoglucanase Gene in *Streptomyces lividans*," Y.-C. Chou, T.B. Vinzant, R.A. Nieves, M.E. Himmel, and S.R. Thomas, Presented as Poster No.60 at the 17th Annual Symposium on Biotechnology for Fuels and Chemicals, Vail, CO, May 1995.
114. "The Commissioning of a Pilot-Scale Reactor for Dilute-Acid Pretreatment," T. Hsu, M. Himmel, J. Farmer, C. Wyman, and M. Berggren, Presented as an oral paper at the 17th Annual Symposium on Biotechnology for Fuels and Chemicals, Vail, CO, May 1995.
115. "Cloning and Expression of Full Length *Trichoderma reesei* Cellobiohydrolase (CBH) I cDNAs in *E. coli*," R.A. Laymon, A. Mohagheghi, M.E. Himmel, and S.R. Thomas, Presented as poster No.80 at the 17th Annual Symposium on Biotechnology for Fuels and Chemicals, Vail, CO, May 1995.
116. "Synergism and Soluble-Sugar Production in Hybrid Cellulase Systems," J.O. Baker, W.S. Adney, R.A. Nieves, S.R. Thomas, and M.E. Himmel, Presented as poster No.57 at the 17th Annual Symposium on Biotechnology for Fuels and Chemicals, Vail, CO, May 1995.
117. "Evaluation of Commercial Cellulase Preparations for Use in Ethanologenic Fermentations of Cellulosic Biomass," R.A. Nieves, W.S. Adney, C.I. Ehrman, S.R. Thomas, and M.E. Himmel, Presented as Poster No.85 at the 17th Annual Symposium on Biotechnology for Fuels and Chemicals, Vail, CO, May 1995.



118. "Advances in Cellulase Research," S.R. Thomas, W.S. Adney, J.O. Baker, Y.-C. Chou, R.A. Laymon, R.A. Nieves, T.B. Vinzant, X. Xiong, and M.E. Himmel, Presented as an oral paper at the 1995 Annual Meeting of the American Society of Microbiology, Washington, DC, May, 1995.
119. "A Novel Survey and Analysis of Commercial Cellulase Preparations Suitable for Biomass Conversion to Ethanol," R.A. Nieves, W.S. Adney, C.I. Ehrman, S.R. Thomas, and M.E. Himmel, Presented as a poster at the 1995 Annual Meeting of AiChE, Boston, MA, June, 1995.
120. "Progress in the Development of Novel Cellulase Production Systems," S.R. Thomas, W.S. Adney, J.O. Baker, Y.-C. Chou, R.A. Laymon, R.A. Nieves, T.B. Vinzant, X. Xiong, and M.E. Himmel, An oral paper given at the Second Biomass of the Americas Symposium, *Energy, Environment, Agriculture, and Industry*, Portland, OR, August, 1995.
121. "*Acidothermus cellulolyticus*, gen. nov., sp. nov.: Utilization of Cellulose at Hot Spring Temperatures," W.S. Adney, R.A. Nieves, S.R. Thomas, A. Mohagheghi, M.P. Tucker, T.B. Vinzant, and M.E. Himmel, An oral paper to be presented at the symposium on *Biodiversity, Ecology, and Evolution of Thermophiles in Yellowstone National Park: An Overview and Issues*, Old Faithful Lodge, September, 1995.
122. "Expression of *Microbispora bispora* Bgl B in *Streptomyces lividans*," X. Xiong, W.S. Adney, T.B. Vinzant, Y.-C. Chou, M.E. Himmel, and S.R. Thomas, The 8th Annual Colorado Biotechnology Symposium organized by CIRB, Colorado State University, Fort Collins, CO, September, 1995.
123. "Progress Toward the Production of Low Cost Cellulase Preparations for Biomass Conversion," M.E. Himmel, J.O. Baker, W.S. Adney, V.L. Putsche, and S.R. Thomas, An oral paper Presented at The 1995 International Chemical Congress of Pacific Basin Societies Symposium on *Pretreatment and Hydrolysis of Biomass*, Honolulu, Hawaii, December, 1995.
124. "Biomass to Ethanol Conversion Technologies in the U.S.," J.R. Mielenz, G.P. Philippidis, Q. Nguyen, C. Hatzis, S.R. Thomas, S.K. Picataggio, J.D. McMillan, R.T. Elander, D. Koepping, F. Parson, C.E. Wyman, and M.E. Himmel, A poster Presented at The 1995 International Chemical Congress of Pacific Basin Societies Symposium, IEA Symposium on *Bioconversion of Lignocellulosics*, Honolulu, Hawaii, December, 1995.
125. "Cellulase Superfolds: Diversity of Structure and Convergence of Function," M.E. Himmel, J.O. Baker, W.S. Adney, R.A. Nieves, and S.R. Thomas, Presented at the 18th Annual Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN, May 1996.
126. "A Membrane Reactor Saccharification Assay to Evaluate the Performance of Cellulases and Substrate Pretreatments under Simulated SSF Conditions," J.O. Baker, W.S. Adney, T.B. Vinzant, Y.-C. Chou, R.A. Nieves, S.R. Thomas, and M.E. Himmel, Presented at the 18th Annual Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN, May 1996.
127. "A Membrane Reactor Saccharification Assay to Evaluate the Performance of Cellulases Under Simulated SSF Conditions," J.O. Baker, W.S. Adney, T.B. Vinzant, C.I. Ehrman, Y.-C. Chou, R.A. Nieves, S.R. Thomas, and M.E. Himmel, Presented at The 9th Annual Colorado Biotechnology Symposium organized by CIRB, Colorado State University, Fort Collins, CO, September, 1996.
128. "Hydrolysis of Cellulose Using Ternary Mixtures of Purified Cellulases," J.O. Baker, C.I. Ehrman, W.S. Adney, S.R. Thomas, and M.E. Himmel, Presented at the Nineteenth Symposium on Biotechnology for Fuels and Chemicals, Colorado Springs, CO, May, 1997.
129. "Formation of Transglycosylation Products During the Enzymatic Saccharification of Corn Biomass to Ethanol," R.A. Nieves, C.I. Ehrman, R.T. Elander, and M.E. Himmel, Presented at the Nineteenth Symposium on Biotechnology for Fuels and Chemicals, Colorado Springs, CO, May, 1997.
130. "Technoeconomic Evaluation of Integrated *Trichoderma reesei* Cellulase Production Technology for Bioethanol Production," David A. Glassner, Robert J. Wooley, Todd B. Vinzant, and Michael E. Himmel, Presented at the Nineteenth Symposium on Biotechnology for Fuels and Chemicals, Colorado Springs, CO, May, 1997.
131. "Aerobic Microbial Isolates Originating from Yellowstone Hot Springs: Survey of Non-cellulosic Polysaccharide Hydrolyzing Ability," S.R. Decker, W.S. Adney, T.B. Vinzant, and M.E. Himmel, Presented at the Nineteenth Symposium on Biotechnology for Fuels and Chemicals, Colorado Springs, CO, May, 1997.
132. "Engineering Effective Cellulase Systems for Bioethanol Production," Michael E. Himmel, William S. Adney, John O. Baker, Rafael A. Nieves, Todd B. Vinzant, and Steven R. Thomas, Presented at the Annual SIM Meeting in Reno, NE, August, 1997.
133. "Engineering Cellulase Systems: Enzyme Modifications For Improved Function," M.E. Himmel, W.S. Adney, J.O. Baker, S.R. Decker, T.B. Vinzant, R.A. Nieves, S. Godbole, and S.R. Thomas. Invited presentation at the Special Symposium *Structure and Function of Microbial Cellulases*, The 98<sup>th</sup> General Meeting of the American Society for Microbiology, Atlanta, GA, May 1998.
134. "Substrate-Specific Induction and Characterization of Cellulases From *Trichoderma reesei*," T.B. Vinzant, W.S. Adney, S.R. Decker, J.O. Baker, R.A. Nieves, and M.E. Himmel, Presented at the 20th Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN, May, 1998.
135. "Interactions Between *Acidothermus cellulolyticus* Endoglucanase I and *Trichoderma reesei* CBH I in the Saccharification of Microcrystalline Cellulose and Dilute-Acid-Pretreated Yellow Poplar," J.O. Baker, W.S. Adney, S.R. Decker, C.I. Ehrman, T.B. Vinzant, and M.E. Himmel, Presented at the 20th Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN, May, 1998.

136. "Isolation and Characterization of *T. reesei* CBH I Cloned in *Pichia pastoris*," S. Godbole, S.R. Thomas, R.A. Nieves, W.S. Adney, T.B. Vinzant, and M.E. Himmel, Presented at the 20th Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN, May, 1998.
137. "Discovery and Comparison of Thermophilic and Mesophilic Polysaccharidases for Industrial Applications," S.R. Decker, W.S. Adney, T.B. Vinzant, and M.E. Himmel, Presented at the 20th Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN, May, 1998.
138. "More Effective, Less Expensive Enzymes for Biomass Conversion Processes," S.R. Thomas, W.S. Adney, J.O. Baker, S. Decker, S. Godbole, M.E. Himmel, R.A. Nieves, M. Ruth, T.B. Vinzant, SIM Meeting, 8/98, Denver, CO.
139. "Improved Cellulases for Bioethanol Production," M.E. Himmel, W.S. Adney, J.O. Baker, S.R. Decker, T.B. Vinzant, R.A. Nieves, and S.R. Thomas, Tenth CIFAR Conference entitled "Enzyme Innovations for Food and Agriculture," UC Davis, Davis, CA, October 9, 1998.
140. "Protein Engineering for Bioethanol Production," M.E. Himmel, W.S. Adney, J.O. Baker, S.R. Decker, T.B. Vinzant, R.A. Nieves, and S.R. Thomas, DOE Laboratory Catalysis Research Symposium, Albuquerque, NM, Feb. 24-25, 1999.
141. "Cellulase Action As Viewed Using An Analytical Membrane-Reactor Assay," J.O. Baker, M.R. King, W.S. Adney, T.B. Vinzant, S.R. Decker, J. Sakon, and M.E. Himmel, Annual ACS Meeting in Anaheim, CA. March 12-15, 1999.
142. "Designing Catalytically Enhanced Endocellulase," J. Sakon, J. McCarter, R. Lovett, W.S. Adney, J.O. Baker, and M.E. Himmel, Annual ACS Meeting in Anaheim, CA. March 12-15, 1999.
143. "Engineering Cellulases: Endoglucanase Modifications For Improved System Function," W.S. Adney, J.O. Baker, S.R. Decker, T.B. Vinzant, R.A. Nieves, S.R. Thomas, and M.E. Himmel, The 21st Symposium on Biotechnology for Fuels and Chemicals, Fort Collins, CO May, 1999.
144. "Preliminary Economic Analysis of Agricultural Enzyme Production for Use in Cellulose Hydrolysis," M.F. Ruth, J.A. Howard, Z. Nikolov, B.S. Hooker, M.E. Himmel, and S.R. Thomas, The 21st Symposium on Biotechnology for Fuels and Chemicals, Fort Collins, CO May, 1999.
145. "Biomass Conversion by *Trichoderma reesei* Cellulase: Analysis by 2-D Gel Electrophoresis," T.B. Vinzant, W.S. Adney, S.R. Decker, M.R. King, J.O. Baker, M.T. Kinter, N.E. Sherman, J.W. Fox, and M.E. Himmel, The 21st Symposium on Biotechnology for Fuels and Chemicals, Fort Collins, CO May, 1999.
146. "Improved Cellulases for Bioethanol Production," M.E. Himmel, W.S. Adney, S.R. Decker, T.B. Vinzant, R.A. Nieves, S.R. Thomas, and J.O. Baker. Gordon Research Conference on "Cellulases and Cellulosomes," Proctor Academy, New Hampshire, July 25-30, 1999.
147. "Improved Cellulases for Bioethanol Production," W.S. Adney, J.O. Baker, S.R. Decker, S.L. McCarter, J. Sheehan, T.B. Vinzant, and M.E. Himmel, Presented at Wood and Cellulose: Building Blocks for Chemicals, Fuels, and Advanced Materials, SUNY College of Environmental Science & Forestry, Syracuse, NY, April 9-11, 2000.
148. "Heterologous Expression of *Trichoderma reesei* CBH I: Effect of Site-Directed Mutations on Expression and Thermostability" S.R. Decker, W.S. Adney, J.O. Baker, S.L. McCarter, T.B. Vinzant, J. Sakon\*, K.L. Barnett, and M.E. Himmel, The 22nd Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN May, 2000.
149. "Site-Directed Mutagenesis of the EI Endoglucanase from *Acidothermus cellulolyticus*," S.L. McCarter, W.S. Adney, J.O. Baker, T.B. Vinzant, R.D. Guckian, S.R. Decker, J. Sakon\*, and M.E. Himmel, The 22nd Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN May, 2000.
150. "Thermophilic Bacteria of the Kamchatka Peninsula as Producers of Carbohydrate Degrading Enzymes," W.S. Adney, M.E. Himmel, I. Andreeva, N. Pechurkina, G. Karpov, and V. Repin, Combined Rocky Mountain and Pacific ACS Regional Meeting, Idaho Falls, Idaho, June 14, 2000.
151. "Development of a Total Cellulytic Activity Assay for High Throughput Screening," Vargas, José Manuel [Presenter], Carlos Flores, Yamir Diaz, Eli Tilén, Edwin G. Rivera, and L. Saliceti-Piazza, Department of Chemical Engineering, University of Puerto Rico, Mayagüez, Puerto Rico; Catalina Flores, Department of Chemical Engineering, Iowa State University, Ames, IA; and R. Nieves, M. Himmel, and S. Decker, National Renewable Energy Laboratory, Golden, CO. Paper No. 164-155, National Student Poster Session, 2000 Annual AIChE Meeting, Los Angeles, CA. November 12-17, 2000.
152. "High-Throughput Screening of Cellulases: Automated Filter Paper Assay and Beyond," Stephen R. Decker\*, Edward W. Jennings, William S. Adney, Todd B. Vinzant, Suzanne L. McCarter, John O. Baker, and Michael E. Himmel, The 23rd Symposium on Biotechnology for Fuels and Chemicals, Breckenridge, CO May, 2001.
153. "Characterization of Starch Degrading Enzymes from a Marine Bacterium. Diana Franqui-Espiet, Govind S. Nadathur\*, William S. Adney, Suzanne L. McCarter, Rafael A. Nieves, Todd B. Vinzant, and Michael E. Himmel, The 23rd Symposium on Biotechnology for Fuels and Chemicals, Breckenridge, CO May, 2001.

154. "Discovery Of Industrial Enzymes From Thermophilic Bacteria Of The Kamchatka Peninsula" W.S. Adney, I. Andreeva\*, N. Pechurkina\*, G. Karpov\*\*, D. Franqui-Espiet, M.E. Himmel, and V. Repin, The 23rd Symposium on Biotechnology for Fuels and Chemicals, Breckenridge, CO May, 2001.
155. Effect of a Single Active-Site-Cleft Mutation upon Product Specificity in a Thermostable Bacterial Cellulase. Tauna R. Rignall, John O. Baker, William S. Adney, Suzanne L. McCarter, Todd B. Vinzant, Steven R. Decker, and Michael E. Himmel, The 23rd Symposium on Biotechnology for Fuels and Chemicals, Breckenridge, CO May, 2001.
156. Substrate Interactions Displayed by a Surface-Modified Endoglucanase from *A. cellulolyticus*. Suzanne L. McCarter, William S. Adney, Todd B. Vinzant, Fannie Posey-Eddy, John O. Baker, Tauna Rignall, Joshua Sakon\*, and Michael E. Himmel., The 23rd Symposium on Biotechnology for Fuels and Chemicals, Breckenridge, CO May, 2001
157. "Cellulase/cellulose interaction: Site directed surface modifications of a family 5 endoglucanase". Suzanne L. McCarter, William S. Adney, Todd B. Vinzant, Fannie Posey-Eddy, John O. Baker, Tauna Rignall, Joshua Sakon, and Michael E. Himmel, SIM Annual Meeting., July 25-30, 2001.
158. "Diversity and Conservation of Relevant GH Families in Well-Studied Cellulolytic Microbes," S.-Y. Ding, W.S. Adney, S.M. McCarter, S.R. Decker, T.B. Vinzant, and M.E. Himmel. Gordon Research Conference on "Cellulases and Cellulosomes," Poster A3, Proctor Academy, New Hampshire, July 25-Aug 3, 2001.
159. "Temperature and Enzyme Loadings as Variables in Cellulase Kinetics," J.O. Baker, Tauna R. Rignall, William S. Adney, Suzanne L. McCarter, Todd B. Vinzant, Steven R. Decker, and Michael E. Himmel. Gordon Research Conference on "Cellulases and Cellulosomes," Proctor Academy, New Hampshire, July 25-Aug 3, 2001.
160. "Enzymatic Depolymerization of Biomass Carbohydrates: New Perspective", M.E. Himmel, Fifth Biomass Conference of the Americas, Orlando, FL, September 17-21, 2001.
161. "Improved Cellulases Through Protein Engineering," M.E. Himmel, J. Brady, J. Sakon, W.S. Adney, S. McCarter, S.R. Decker, T.B. Vinzant, T. Rignall, J.O. Baker, C. Skopec, and S.-Y. Ding. A. Payen Special Symposium Honoring Liisa Viikari, Application of Enzymes to Lignocellulosics, 223<sup>rd</sup> Annual ACS Meeting, Orlando, FL. April 7-10, 2002.
162. "Utility of Cellobiose Dehydrogenase in the Automated Measurement of Cellulolytic Activity, Stephen R. Decker, Edward W. Jennings, Todd B. Vinzant, William S. Adney, and Michael E. Himmel, The 24nd Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN May, 2002.
163. "New Glycosyl Hydrolases from *Acidothermus cellulolyticus*, Shi-You Ding, William S. Adney, Stephen R. Decker, John O. Baker, Ed Jennings, Todd B. Vinzant, and Michael E. Himmel, The 24nd Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN May, 2002.
164. "Proteases and the Extracellular Lignin Depolymerase Activity from *Trametes cingulata*, Yi-ru Chen, Todd B. Vinzant,\* Stephen R. Decker,\* Ed Jennings,\* Michael E. Himmel,\* and Simo Sarkanen, The 24nd Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN May, 2002.
165. "Changes in Cellulose Morphology of Pretreated Yellow Poplar During Enzymatic Hydrolysis," Mark Davis, John Baker Tauna Rignall, and Michael Himmel, The 24nd Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN May, 2002.
166. "Expression of Endoglucanase E1 in Transgenic Duckweed *Lemna minor*," Ye Sun, William S. Adney, Ben A. Bergmann, Jiayang Cheng, Stephen R. Decker, Shelby Freer, Michael E. Himmel, Yufuko Nishimura, Christopher D. Skory, Anne-Marie Stomp, Steven Thomas, Brent Tisserat, Yuri T. Yamamoto, The 24th Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN May, 2002.
167. "Conjugation of Self-Assembling Proteins to CdSe/ZnS Quantum Dots. S.-Y. Ding, G. Rumbles, W.S. Adney, M. Jones, J. Nedeljkovic, M.P. Tucker, A.J. Nozik, and M.E. Himmel, 25<sup>th</sup> DOE OS Photochemistry Program Meeting, Warrenton, VA, June 9-12, 2002.
168. "QD-Bioconjugates Assembled Using Genetically Modified Proteins," S.-Y. Ding, M.E. Himmel, W.S. Adney, M.P. Tucker, J. Wall, J. Nedeljkovic, O.I. Micic, M. Jones, A.J. Nozik and G. Rumbles, To be presented at the MRS Symposium H: Bio-Inspired Nanoscale Hybrid Systems, San Francisco, CA, Spring 2003.
169. "Heterologous Expression, Purification, and Characterization of the Glycosyl Hydrolase Family 7 Cellobiohydrolases from *Trichoderma reesei* and *Penicillium funiculosum*," William S. Adney, Yat-Chen Chou, Stephen R. Decker, John O. Baker and Michael E. Himmel, To be presented as an oral paper at the 225<sup>th</sup> Annual American Chemical Society Meeting, New Orleans, LA, March 22-27, 2003.
170. "Molecular Mechanics Modeling of *Trichoderma reesei* Cel7A," T. Rignall, C. McCabe, D. Wood, J. Brady, and M. Himmel, To be presented at the Foundations of Molecular Modeling and Simulation (FOMMS) Annual meeting, Aspen, CO. July 6-11, 2003.
171. "Heterologous Expression, Purification, and Characterization of a Cellobiohydrolase from *Penicillium funiculosum*," Yat-Chen Chou, William S. Adney, Stephen R. Decker, John O. Baker and Michael E. Himmel, To be presented at the 25nd Symposium on Biotechnology for Fuels and

Chemicals, Breckenridge, CO. May, 2003.

172. "The Effect of Lignin Modifying Enzymes on the Molecular Weight Distribution of kraft Lignin," Stephen R. Decker, Aarti Gidh, Michael E. Himmel, Todd B. Vinzant, To be presented at the 25nd Symposium on Biotechnology for Fuels and Chemicals, Breckenridge, CO. May, 2003.
173. "Increased Thermal Tolerance of *T. fusca* beta-Glucosidase *via* Directed Evolution," Eric E. Jarvis, William S. Adney, Stephen R. Decker, John O. Baker, Michael E. Himmel, To be presented at the 25nd Symposium on Biotechnology for Fuels and Chemicals, Breckenridge, CO. May, 2003.

## Organization/Support of Scientific Meetings

1. **Chaired Poster Session:** The 9th Symposium on Biotechnology for Fuels and Chemicals, Boulder, CO, May, 1987.
2. **Chaired Session:** "Bioprocessing Technology," J.R. Mattoon, co-chair, the Second Annual Colorado Biotechnology Symposium, Boulder, CO, September, 1989.
3. **Organized and Chaired International Symposium:** "Enzymes in Biomass Conversion," G. Leatham, co-chair, American Chemical Society (Cellulose, Paper, and Textile Div.), Boston, MA, June, 1990.
4. **Chaired Session:** "Bioprocessing Research," J.A. Doncheck, co-chair, the Thirteenth Symposium on Biotechnology for Fuels and Chemicals, Colorado Springs, CO, May, 1991.
5. **Organized and Chaired International Symposium:** "Biocatalyst Design for Stability and Specificity," G. Georgiou, co-chair, American Chemical Society (Biochemical Technology Division)/Fourth Chemical Congress Combined Meeting, New York, NY, August, 1991.
6. **Chaired Session at Symposium:** "Cellulase Biochemistry," the Cellulose '91 meeting organized by the American Chemical Society and Cellucon Conferences, New Orleans, LA, December, 1991.
7. **Organized and Chaired International Symposium:** "Bioconversion for Fuels", R. Overend, co-chair, the 1993 Annual American Chemical Society Meeting (Biotechnology Secretariat), Denver, CO, February, 1993.
8. **Organized and Chaired International Symposium:** "Applications of Industrial Biotechnology," S. Thomas, co-chair, the 1994 Annual Chemical Society Meeting (Biotechnology Secretariat and Cellulase, Paper, and Textile Division), San Diego, CA, March, 1994.
9. **Co-Chaired Session at Symposium:** "The symposium on Advances in the Bioconversion of Lignocellulosics," The 1994 Annual American Chemical Soc. Meeting, San Diego, CA, March, 1994.
10. **Co-Organized International Workshop:** "U.S. DOE - National Renewable Energy Laboratory sponsored workshop on Nomenclature of Cellulases and Other Related Enzymes," Lake Tahoe, CA, June, 1994.
11. **Organized Special Topics Sessions at International Symposium:** "The 17th Symposium on Biotechnology for Fuels and Chemicals, Vail, CO, May, 1995.
12. **Co-Chaired Session at International Symposium:** Session on "Applied Biotechnology" at the 19th Symposium on Biotechnology for Fuels and Chemicals, Colorado Springs, CO, May 4-8, 1997.
13. **Co-Chaired Session at International Symposium:** Session on "Enzyme Processes and Enzyme Production" at the 20th Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN, May 3-7, 1998.
14. **Organized and Chaired International Symposium:** "Glycosyl Hydrolases for Biomass Conversion," J. Saddler, co-chair, the 1999 Annual Chemical Society Meeting (Cellulase, Paper, and Textile Division), Anaheim, CA, March, 1999.
15. **Vice-Chair:** 2<sup>nd</sup> Annual Gordon Research Conference on "Cellulases and Cellulosomes," Procter, NH, July, 2001.
16. **Chair:** 3<sup>rd</sup> Annual Gordon Research Conference on "Cellulases and Cellulosomes" to be held in Procter, NH, July, 2003.

**William S. Adney**  
Biotechnology Division for Fuels and Chemicals  
National Bioenergy Center  
National Renewable Energy Laboratory  
1617 Cole Blvd, Golden, CO 80401

**Education:**

Institution	Degree	Year	Field
Colorado State University	B.S.	1976	Microbiology
Colorado State University	M.S	1978	Microbiology

**Professional Experience:**

1999 - present	Senior Scientist, Microbiology, National Renewable Energy Laboratory
1987-1998	Staff Scientist, Microbiology, National Renewable Energy Laboratory
1979 -1987	Microbiologist B, Diagnostic Laboratory, Department of Pathology, Colorado State University

**PROFESSIONAL ASSOCIATIONS AND HONORS**

- Specialist in Microbiology Certification by the American Society for Clinical Pathologists
- 1998 - NREL/MRI/DOE Staff Award for Outstanding Team Performance
- 2002 - NREL/MRI/DOE Staff Award for Outstanding Individual Performance
- Member of the American Chemical Society
- Member of the American Society of Microbiology
- Member of the American Biological Safety Association

**Qualifications and Experience**

Extensive knowledge and practical experience in the following areas:

- Protein Purification and Characterization
- Enzymatic Biomass Hydrolysis and Cellulase Kinetics
- Eukaryotic and Prokaryotic Recombinant Protein Expression Technologies
- Industrial, Environmental and Clinical Bacteriology
- Industrial and Clinical Mycology

**Relevant Publications:** Authored or co-authored 49 peer reviewed journal and symposium publications and seven patents.

- "Crystal Structure of Thermostable Family 5 Endocellulase EI from *Acidothermus cellulolyticus* in Complex with Cellotetraose," J. Sakon, **W. Adney**, M.E. Himmel, S. Thomas, and P. Karplus, Biochemistry 35, 10648-10660 (1996).
- "A Membrane-Reactor Saccharification Assay to Evaluate the Performance of Cellulases Under Simulated SSF Conditions," J.O. Baker, T.B. Vinzant, C.I. Ehrman, **W.S. Adney**, and M.E. Himmel, Appl. Biochem. Biotechnol., 63-65, 585-595, (1997).
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- "Advanced Bioethanol Production Technologies: A Perspective", M.E. Himmel, **W.S. Adney**, J.O. Baker, R. Elander, J.D. McMillan, R.A. Nieves, J. Sheehan, S.R. Thomas,

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- "Investigation of the Cell Wall Loosening Protein Expansin as a Possible Additive in the Enzymatic Saccharification of Lignocellulosic Biomass," J.O. Baker, M.R. King, **W.S. Adney**, S.R. Decker, T.B. Vinzant, S.L. Lantz, R.E. Nieves, S.R. Thomas, L.-C. Li, D.J. Cosgrove, and M.E. Himmel, Appl. Biochem. Biotechnol. 84-86, 217-223, (2000).
- "Fingerprinting *Trichoderma reesei* Hydrolases in a Commercial Cellulase Preparation," T.B. Vinzant, **W.S. Adney**, S.R. Decker, J.O. Baker, M.T. Kinter, N.E. Sherman, J.W. Fox, and M.E. Himmel, Appl. Biochem. Biotechnol. 99-107 (2001).
- "Exploration of the Cellulose Surface Binding Properties of A. *cellulolyticus* Cel5A by Site Specific Mutagenesis," S.L. McCarter, **W.S. Adney**, T.B. Vinzant, F. Posey-Eddy, S.R. Decker, J.O. Baker, J. Sakon, and M.E. Himmel, Appl. Biochem. Biotechnol. 2001, In press.
- "Effect of a Single Active-Site-Cleft Mutation upon Product Specificity in a Thermostable Bacterial Cellulase," T.R. Rignall, J.O. Baker, S.L. McCarter, **W.S. Adney**, T.B. Vinzant, S.R. Decker and M.E. Himmel, Appl. Biochem. Biotechnol. 2001, In press.
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# WILLIAM SCOTT ADNEY

## PUBLICATION LIST

### *JOURNAL AND SYMPOSIUM PAPERS*

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2. "Experimental Production of Pasteurellosis in Goats", W.S. Adney, L.H. Lauerman, C.V. Kimberling, T.A. Ngatia and L. Johnson, In *Proceedings of the Third International Conference on Goat Production and Disease*, (1982).
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4. "Aspects of Bacteriology and Endocrinology of Cows with Pyometra and Retained Fetal Membranes", J.D. Olson, L. Ball, R.G. Mortimer, P.W. Farin, W.S. Adney and M. Huffman, Am. J. Vet. Res., **45**, 2251-2255 (1984).
5. "Evaluation of the RapID-ANA System for Identification of Anaerobic Bacteria from Veterinary Clinical Specimens", R.L. Jones and W.S. Adney, In *American Association Veterinary Laboratory Diagnosticians 27<sup>th</sup> Annual Proceedings*, 305-310 (1984).
6. "Evaluation of the RapID-ANA System for Identification of Anaerobic Bacteria of Veterinary Origin", W.S. Adney and R.L. Jones, J. Clin. Microbiol., (1985).
7. "Anaerobic Infections in Dogs and Cats: Review of Clinical Cases and Response to Therapy", S.W. Dow, R.L. Jones and W.S. Adney, J. Am. Vet. Med. Assoc., **189**, 930-934 (1986).
8. "Isolation of *Clostridium difficile* and Detection of Cytotoxin in the Feces of Diarrheic Foals in the Absence of Antimicrobial Treatment", R.L. Jones, W.S. Adney and R.K. Shideler, J. Clin. Microbiol., **25**, 1225-1227 (1987).
9. "Evaluation of Quantum II Microbiology System for Identification of Gram-Negative Bacteria of Veterinary Origin", R.L. Jones, W.S. Adney, M.A. Davis, H. VonByrn and G. Thompson, J. Clin. Microbiol., **25**, 2071-2074 (1987).
10. "Anaerobic High Solids Fermentation of Processed Municipal Solid Wastes for the Production of Methane", C.J. Rivard, M.E. Himmel, T.B. Vinzant, W.S. Adney, C.E. Wyman and K. Grohmann, Applied Biochem. Biotech., **20/21**, 461-47 (1989).
11. "Detection of Extracellular Hydrolytic Enzymes in the Anaerobic Digestion of Municipal Solid Waste", W.S. Adney, C.J. Rivard, K. Grohmann, and M.E. Himmel, Biotechnol. Appl. Biochem., **11**, 387-400 (1989).
12. "Waste to Energy: Nutrient Requirements for Aerobic and Anaerobic Digestion of Processed Municipal Solid Waste", C.J. Rivard, T.B. Vinzant, W.S. Adney, and K. Grohmann, J. Environ. Health., **52**, 96-99 (1989).
13. "Characterization of Polysaccharidase Activity Optima in the Anaerobic Digestion of Municipal Solid Waste", W.S. Adney, C.J. Rivard, K. Grohmann, and M.E. Himmel, Biotech. Letters, **3**, 207-210 (1989).
14. "Anaerobic Digestion of Processed Municipal Solid Waste Using a Novel High Solids Reactor: Maximum Solids Levels and Mixing Requirements", C.J. Rivard, M.E. Himmel, T.B. Vinzant, W.S. Adney, C.E. Wyman, and K. Grohmann, Biotechnol. Lett., **12(3)**, 235-240 (1990).



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23. "Effects of Natural Polymers Acetylation on the Anaerobic Bioconversion to Methane and Carbon Dioxide", C.J. Rivard, W.S. Adney, M.E. Himmel, D.J. Mitchell, T.B. Vinzant, K. Grohmann, L. Moens, and H. Chum, Appl. Biochem. Biotech., **34/35**, 217-231 (1992).
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25. "Isolation and Characterization of Two Forms of -D-Glucosidase from *Aspergillus niger*", M.E. Himmel, W.S. Adney, D.J. Mitchell, and J.O. Baker, Appl. Biochem. Biotechnol., **39/40**, 213-225 (1993).
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27. "Purification and Characterization of Acetyl-Xylan Esterase from *Aspergillus niger*," J.C. Linden, M. Samara, M. Pecs, E. Thomas, M. Joy, W.S. Adney, and M.E. Himmel, Appl. Biochem. Biotechnol., **45/46**, 383-393 (1994).
28. "Cellulase Assays: Methods from Empirical Mathematical Models," W.S. Adney, C.I. Ehrman, J.O. Baker, S.R. Thomas, And M.E. Himmel, In *Enzymatic Conversion of Biomass For Fuels Production*, (M.E. Himmel, J.O. Baker, and R.P. Overend, eds.), ACS Series **566**, American Chemical Society: Washington, DC, 1994, pp. 218-235.

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30. "Comparison of Protein Contents of Cellulase Preparations in a Worldwide Round-Robin Assay," W.S. Adney, A. Mohagheghi, S.R. Thomas, and M.E. Himmel, In *Enzymatic Degradation of Insoluble Polysaccharides*, (J.N. Saddler, and M. Penner, eds.), ACS Series **618**, American Chemical Society: Washington, DC, 1995, pp. 256-271.
31. "Low Molecular Weight Thermostable -D-glucosidase from *Acidothermus cellulolyticus*," W.S. Adney, M.P. Tucker, R.A. Nieves, S.R. Thomas, and M.E. Himmel, *Biotech. Lett.*, **17**, pp. 49-54 (1995).
32. "Initial Approaches to Artificial Cellulase Systems for Conversion of Biomass to Ethanol", S.R. Thomas, R.A. Laymon, Y.-C. Chou, M.P. Tucker, T.B. Vinzant, W.S. Adney, J.O. Baker, R.A. Nieves, J.R. Mielenz, and M.E. Himmel, In *Enzymatic Degradation of Insoluble Polysaccharides*, (J.N. Saddler, and M. Penner, eds.), ACS series **618**, American Chemical Society: Washington, DC, 1995, pp. 208-236.
33. "Synergism in Binary Mixtures of Bacterial and Fungal Cellulases: Endo/Exo, Exo/Exo, and Endo/Endo Interactions." J.O. Baker, W.S. Adney, R.A. Nieves, S.R. Thomas, and M.E. Himmel, In *Enzymatic Degradation of Insoluble Polysaccharides*, (J.N. Saddler, and M. H. Penner, eds.), ACS Series **618**, American Chemical Society: Washington, DC, 1995, pp. 113-141.
34. "Cloning and Expression of Full-Length *Trichoderma reesei* Cellobiohydrolase I cDNA's in *Escherichia coli*," Robert A. Laymon, William S. Adney, Ali Mohagheghi, Michael E. Himmel, and Steven R. Thomas, *Appl. Biochem. Biotechnol.*, **57/58**, 389-397 (1996).
35. "Crystal Structure of Thermostable Family 5 Endocellulase E1 from *Acidothermus cellulolyticus* in Complex with Cellotetraose," J. Sakon, W. S. Adney, M.E. Himmel, S.R. Thomas, and P.A. Karplus, *Biochemistry* **35**, 1996, pp 10648-10660.
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37. "A membrane-Reactor Saccharification Assay to Evaluate the Performance of Cellulases Under Simulated SSF Conditions," John O. Baker, T. B. Vinzant, C.I. Ehrman, W.S. Adney, and M. E. Himmel, *Appl Biochem. Biotechnol.*, **63/65**, 585-595, (1997).
38. "Polysaccharide Hydrolase Folds: Diversity of Structure and Convergence of Function," M.E. Himmel, J. Sakon, J.O. Baker, W.S. Adney, P.A. Karplus, and S.R. Thomas, *Appl. Biochem. Biotechnol.*, **63/65**, 315-326, (1997).
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41. "Advanced Bioethanol Production Technologies: A Perspective", M.E. Himmel, W. S. Adney, J.O. Baker, R. Elander, J.D. McMillan, R.A. Nieves, J. Sheehan, S.R. Thomas, T.B. Vinzant, and M. Zhang, In *Fuels and Chemicals from Biomass*; (J. Woodward and b. Saha, eds.) ACS Series **666**, American Chemical Society: Washington, DC, 1997, 2-45.
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45. "Investigation of the Cell Wall Loosening Protein Expansin as a Possible Additive in the Enzymatic Saccharification of Lignocellulosic Biomass," J.O. Baker, M.R. King, W.S. Adney, S.R. Decker, T.B. Vinzant, S.E. Lantz, R.E. Nieves, S.R. Thomas, L.-C. Li, D.J. Cosgrove, and M.E. Himmel, *Applied Biochemistry and Biotechnology*, (1999) In Press.
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#### MEETING ABSTRACTS AND PROCEEDINGS

1. "Laboratory Model for Pasteurella Pneumonia", W.S. Adney, Oral paper presented to the 2<sup>nd</sup> Annual Western Conference for Food Animal Veterinary Medicine. Fort Collins, CO. (1981).
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6. "High Solids Anaerobic Digestion of MSW for the Production of Methane: Successful Operation of a Novel Laboratory-Scale, Continuously Mixed Reactor", C.J. Rivard, W.S. Adney, T.B. Vinzant, M.E. Himmel, C.E. Wyman, and K. Grohmann, The 1989 ASM Annual Meeting, Poster O50, New Orleans, LA, (1989).

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14. "Anaerobic Biodegradation of Starch Acetate and Cellulose Acetate Plastic Copolymers", C.J. Rivard, T.B. Vinzant, W.S. Adney, M.E. Himmel, K. Grohmann, National Corn Growers Association, St. Louis, MO, June (1990).
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